



Hot spices

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Hot Spices

Effects on appetite, Energy Intake and Sensory
Properties of a Meal

PhD thesis by

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Title:

Hot Spices - Effects on appetite, Energy Intake and Sensory Properties of a Meal.

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Preface and Acknowledgements

The present PhD-project is supported by the Directorate for Food, Fisheries and Agri Business and by the Danish Meat Research Institute (DMRI) under the law of innovation (Innovationsloven). The project is part of the collaboration 'Meat as part of a meal' formed between LIFE, University of Copenhagen, DMRI, a group of food companies such as Agrova, Tican Foods, Danish Crown, Kryta and Kram Food Service to do research and development work to promote the nutritional and culinary quality of centrally prepared meals with pork. The research has mainly been carried out in the Sensory Science Group, Department of Food Science, Faculty of Life Sciences (LIFE), University of Copenhagen, Denmark under supervision of Per Møller (paper II and III). One study was performed at the DMRI, Roskilde, Denmark under supervision of Margit Dall Aaslyng (paper I). Experiences with a foreign research environment were gained through a six month stay at Maastricht University, the Netherlands where I had the pleasure of working with Margriet Westerterp-Plantenga and her competent employees at the Department of Human Biology (paper IV).

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Helene Christine Reinbach

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Abbreviations

BP	blood pressure
BMR	basic metabolic rate
BMI	body mass index
C	capsicum
CHO	carbohydrate
CCK	cholecystokinin
CNS	central nervous system
DIT	diet-induced thermogenesis
DMRI	Danish Meat Research Institute
EGCG	epigallocatechin-3-gallate
EE	energy expenditure
F	fat
FFA	free fatty acids
FFM	fat free mass
FM	fat mass
GI	gastrointestinal
GLP-1	Glucagon-like peptide 1
GLP-2	Glucagon-like peptide 2
HC	high carbohydrate
HF	high fat
HFC	high-frequency component
HR	heart rate
ISI	interstimulus interval
LED	low energy diet
LDL	low-density lipoprotein
LFC	low-frequency component
Min	minute(s)
NTS	nucleus tractus solitarius
OFC	orbitofrontal cortex
PP	pancreatic polypeptide
P	protein
PYY	Peptide YY
REE	resting energy expenditure
RQ	respiratory quotient
SHU	Scoville heat units
SIR	stimulus-induced recovery
SNS	sympathetic nervous system
SSS	sensory-specific satiety
TG	triacylglycerol
VAS	visual analogue scales
VLED	very low energy diet
WGA	wheat germ agglutinin
Wk	week(s)
WHO	World Health Organization
WM	weight maintenance
WOF	warmed-over flavour

Summary

The aim of this PhD-project has been to investigate how trigeminal stimuli affect the sensory properties of foods as well as look at how hot spices and bioactive ingredients affect appetite and energy intake.

Trigeminal stimuli are believed to enhance overall flavour of foods however irritants, such as capsaicin, have been shown to stimulate and suppress tastes whereas the effects of capsaicin on complex flavours have been less explored. The first two articles presented in the thesis investigate how oral burn is affected by respectively texture (paper I) and heat perception (paper II) as well as how oral burn affects the flavour of chilli spiced pork patties (paper I). Chilli was found to suppress meat flavour, while the two textures, respectively 0.5 and 5 % flour, had no effect on meat flavour or oral burn. Based on the current knowledge we believe that suppression of meat flavour was mainly caused by interactions between olfactory and trigeminal stimuli via either a central neural integration of sensory input or via peripheral effects; however, a cognitive phenomenon in which the dominating oral burn draws attention away from other sensations might also contribute to the suppression of meat flavour. Oral burn has been shown to increase linearly with temperature however we found a non-linear temperature dependency of oral burn. Chilli spiced pork patties served at 38 °C were slightly more intense than at 67 °C and lowest intensity of oral burn was found when served at 8 °C. These findings could indicate that binding of capsaicin to its receptor might be temperature dependent with optimum around 37 °C or that taste and texture complexity of the pork patties have reduced the impact of temperature on oral burn. A study was then conducted to reveal if capsaicin can suppress tastes by interacting with the gustatory sense (manuscript I). The ratio between ERK1+2 protein and its phosphorylated form was measured in rat taste cells by immunostaining and used as an indicator for activity in bitter and sweet taste signalling pathways. Sucrose alone was found to activate taste cells more than the combination of sucrose and capsaicin suggesting that capsaicin can suppress activation in sweet taste signalling pathways in rat taste cells. However filiform papillae bended when capsaicin was present suggesting that capsaicin destroys the epithelium or that the tissue has been spoiled during preparation of the samples. Therefore this assay needs to be optimized further before valid conclusions can be drawn.

Bioactive ingredients such as capsaicin (chilli pepper), caffeine (coffee and tea) and catechins (green tea) have in several animal and human studies shown to have beneficial effects on energy expenditure, substrate oxidation and body weight suggesting these ingredients as potential weight-loss candidates. However less is known about how bioactive ingredients affect energy intake and appetite when given individually and in combination. Paper III investigates how hot spices (capsaicin, horseradish, ginger, mustard and wasabi) served in tolerable doses with a fixed dinner meal affect appetite, sensory specific desires, mood and energy intake at a succeeding ad libitum buffet. Hot spices had only weak effects on energy intake, appetite and general mood suggesting that higher doses or several exposures of hot spices are needed to promote energy metabolism, energy intake and appetite. Observing people only at dinner time might be too short a time to capture the full effects of hot spices on appetite and energy intake. Therefore appetite and energy regulating effects of bioactive ingredients (chilli, green tea, CH-19 sweet pepper and the combination of capsaicin and green tea) were studied when added to three daily meals during respectively three weeks of negative and three weeks of positive energy balance. CH-19 sweet pepper and a combination of capsaicin and green tea were found to reduce energy intake during positive energy balance. Interestingly capsaicin and green tea affected several appetite measures (hunger, fullness and satiety) and combining capsaicin and green tea caused even greater hunger suppressive and satiety enhancing effects than when administered individually, yet stronger effects in negative than in positive energy balance were observed. These findings indicate that energy balance affects how capsaicin, green tea and the combination of the two influence appetite. Bioactive ingredients might furthermore induce greater effects on energy intake when used in combinations and when used in positive energy balance. Finally we demonstrated that chilli and mustard respectively changed desire to eat sweet and salty stimuli whereas capsaicin, green tea and the combination of capsaicin and green tea reduced desire to eat fatty, salty and hot stimuli. These findings indicate that bioactive ingredients can change food desires, which might influence food choice and energy intake. However taking these and other findings into account, it is fair to assume that we have not fully explored the potential of using bioactive ingredients for prevention or treatment of obesity and the effects of bioactive ingredients should therefore be studied further, both individually and in combination, to reveal if they affect appetite, energy intake and body weight in the long term and by which mechanism these effects can be explained. Furthermore a better understanding of how hot spices affect the overall

flavour of foods and how sensory properties of spicy foods affect food desires, appetite, energy intake and digestive behaviour could be useful in developing tastier and healthier foods with greater satiating and satisfying properties.

Sammendrag

Hovedformålet med dette ph.d.-projekt var at undersøge hvordan trigeminale stimuli påvirker fødevarers sensoriske egenskaber samt at udforske hvordan stærke krydderier påvirker mæthedsfornemmelse og energiindtag.

Trigeminale stimuli menes at øge den overordnede smagsoplevelse af mad, og irriteranter såsom capsaicin har vist sig både at stimulere og at undertrykke grundsmagene, mens få studier har undersøgt hvordan irriteranter påvirker lugt. I de to første artikler i afhandlingen undersøges det, hvordan den brændende fornemmelse fra chili påvirkes af tekstur (artikel I) og temperatur (artikel II), samt hvordan den brændende fornemmelse påvirker smagen af chili-frikadeller (artikel I). Chili viste sig at undertrykke kødsmag, mens variationer i tekturen, henholdsvis 0,5 og 5 % mel, ikke påvirkede kødsmag eller den brændende fornemmelse. Vi foreslår, at kødsmag undertrykkes pga. interaktioner mellem lugt-, smags- og trigeminale stimuli, der kan foregå via central integration af nerveaktivitet fra sanserne eller via perifere mekanismer. Dog kan kognitive fænomener, hvor den brændende fornemmelse fjerner fokus fra andre sanseindtryk, også bidrage til at undertrykke kødsmagen. Den brændende fornemmelse har tidligere vist sig at afhænge lineært af temperatur. Vi fandt dog en ikke-lineær sammenhæng mellem temperatur og den brændende fornemmelse. Chili-frikadeller serveret ved 38 °C var mere brændende end ved 67 °C og mindst brændende ved 8 °C. Dette kunne indikere, at bindingen mellem capsaicin og dets receptor kunne være temperaturafhængig med optimum omkring 37 °C eller at tekstur- og smagskompleksiteten i chili-frikadellerne kunne have reduceret temperatúrafhængigheden af den brændende fornemmelse. Et studie blev derefter udført for at undersøge om capsaicin kan undertrykke grundsmagene pga. interaktion mellem smag- og den trigeminale sans (manuskript I). Forholdet mellem ERK1+2 protein og dets fosforylerede form blev målt i smagsceller fra rotter vha. immunfarvning og blev brugt som indikator for aktivitet i signaltransduktionsvejen for bitter og sød smag. Sød smag aktiverede smagsceller mere end kombinationen af sød smag og capsaicin, hvilket kunne indikere, at capsaicin undertrykker signaltransduktion for sød smag i smagsceller fra rotter. Dog fik filiforme papiller en bøjet form ved tilstedeværelse af capsaicin, hvilket kunne skyldes, at capsaicin ødelægger epitelet eller at tunge vævet ødelægges under prøvetilberedningen, hvorfor dette assay skal optimeres yderligere for at give valide resultater.

Bioaktive ingredienser, såsom capsaicin fra chili, koffein fra kaffe og te, samt catechiner fra grøn te, har vist sig at have gavnlig effekt på energiomsætning, substrat oxidation og kropsvægt hos både dyr og mennesker, hvorfor disse stoffer kunne have potentiale som vægttabsfremmende midler. Der vides dog ikke meget om, hvordan bioaktive ingredienser påvirker appetit og energiindtag, når de indtages alene eller i kombination med andre bioaktive stoffer. Artikel III undersøger, hvordan stærke krydderier (chili, peberrod, ingefær, sennep og wasabi) påvirker appetit, humør, lysten til sure, søde, fede, bitre, salte og stærke stimuli samt ad libitum energiindtaget, når de indtages i tolererede mængder med et portionsanrettet måltid. Stærke krydderier havde kun svag indflydelse på appetit, energiindtag og humør, hvilket foreslår, at større doser eller flere gentagne stimuleringer med stærke krydderier er nødvendige for at fremme energiomsætning, energiindtag og appetit. Desuden kunne det tænkes, at effekterne af stærke krydderier ikke studeres fuldt ud, når forsøget kun strækker sig over et måltid. Derfor undersøgte vi, hvordan bioaktive ingredienser, såsom capsaicin, grøn te, CH-19 sød peber og kombinationen af capsaicin og grøn te påvirker appetit og energiindtag, når de indtages med tre daglige måltider i henholdsvis positiv og negativ energibalance. CH-19 sød peber og kombinationen af capsaicin og grøn te reducerede energiindtag under positiv energibalance. Capsaicin og grøn te havde interessante effekter på flere appetitparametre (sult, fyldthed og mæthed) og kombinationen af capsaicin og grøn te forstærkede mæthedsfornemmelsen og reducerede sult, set i forhold til effekterne af de enkelte bioaktive ingredienser. De appetitregulerende effekter af capsaicin og grøn te var mest markante under negativ energibalance. Dette indikerer, at energibalancen påvirker den appetitregulerende effekt af capsaicin og grøn te, samt at bioaktive stoffers effekt på energiindtaget forøges, når de indtages i kombination og under positiv energibalance. Ydermere viste vi, at chili og sennep i et måltid ændrede henholdsvis lysten til søde og salte stimuli, hvorimod indtagelse af capsaicin, grøn te og kombinationen af de to ingredienser, tre gange dagligt, formindskede lysten til fede, salte og stærke stimuli. Dette kunne indikere, at bioaktive ingredienser kan ændre lysten til bestemte fødevarer stimuli, hvilket kunne tænkes at have betydning for fødevalg og energiindtag. Ud fra disse og andres observationer antager vi, at bioaktive ingrediensers effekter og deres anvendelsesmuligheder indenfor forebyggelse og behandling af fedme endnu ikke er udforsket til bunds og de gavnlige effekter af de enkelte bioaktive ingredienser og kombinationer af disse kunne derfor udforskes yderligere for at undersøge, om de påvirker appetit, energiindtag og kropsvægt efter

længere tids indtagelse samt undersøge hvilke mekanismer, der kan forklare de observerede effekter. Ydermere er der brug for større forståelse for, hvorledes stærke krydderier påvirker smag, og hvordan stærke fødevarers sensoriske egenskaber påvirker lyst, appetit, energiindtag og fordøjelse, da denne viden kan være nyttig i udviklingen af mere velsmagende og sundere mad med mere mættende og tilfredsstillende egenskaber.

Introduction

Background

Convenience food plays a larger role in the nutrition of the Danes since we have greater accessibility and opportunity to bring home fast food and convenience meals and to have ready-to-eat lunch at work or in school. For elderly people meals are offered and brought to their homes when they are too sick to manage cooking themselves. However it is a challenge for catering companies to manufacture meals on the large scale that have an appealing flavour and appearance and that can meet the nutritional needs for people suffering of sickness and loss of appetite as well as satisfy overweight people who want to loose weight and prevent weight gain.

Gastronomy, which is based on long traditions on how to compose and prepare meals with meat, vegetables, gravy, bread and salads, has led to great knowledge about which ingredients that go well together; however very little is known about how components in a meal interact. Therefore it is necessary to get a better understanding of how meals should be composed and prepared, how different taste, flavour and irritant components in a meal interact and how they influence the sensory perception of the meal.

Bioactive ingredients such as capsaicin (chilli pepper), caffeine (coffee and tea), catechins (green tea) have in several animal and human studies shown to increase energy expenditure (EE) and to reduce energy intake and appetite when given individually or in combination. These well recognized health beneficial effects suggest these components as potential weight-loss candidates. Obesity is now an acknowledged problem within all age groups of the Danish population which emphasises the urgent need for development of novel weight loss and weight maintenance strategies.

Aims and Objectives

The overall focus of the project ‘Meat as part of a meal’ was on doing research and development work to promote the nutritional and culinary quality of future meals prepared with pork on the large scale at catering or food service companies. The aim of this PhD project was to develop healthy low fat meals with hot spices to investigate how trigeminal stimuli affect the sensory properties of meals as well as look at how bioactive ingredients affect appetite and energy intake. Outcomes from these studies could reveal if bioactive ingredients such as hot spices and green tea could have any potential as weight loss agents and could help in developing

healthier and more satisfying convenience meals in the future for people who want to prevent weight gain or aid weight loss. Based on these aims, the PhD project was divided into two major parts, Part I: ‘Sensory Properties of Spicy Meals’ and Part II: ‘Spicy Meals, Appetite and Energy intake’, which were further divided into several underlying research questions (see bullet points in figure 1.1).

Part I Sensory Properties of Spicy Meals

1. How do components in spicy meals interact?
 - How do hot spices affect taste and flavour perception?
 - Do hot spices suppress taste and smell?
2. Which mechanisms can explain interaction between gustatory, olfactory and trigeminal stimuli?

Part II Spicy Meals, Appetite and Energy intake

3. Do hot spices affect appetite and energy intake?
4. Which physiological mechanisms can explain effects of hot spices on energy intake and appetite?

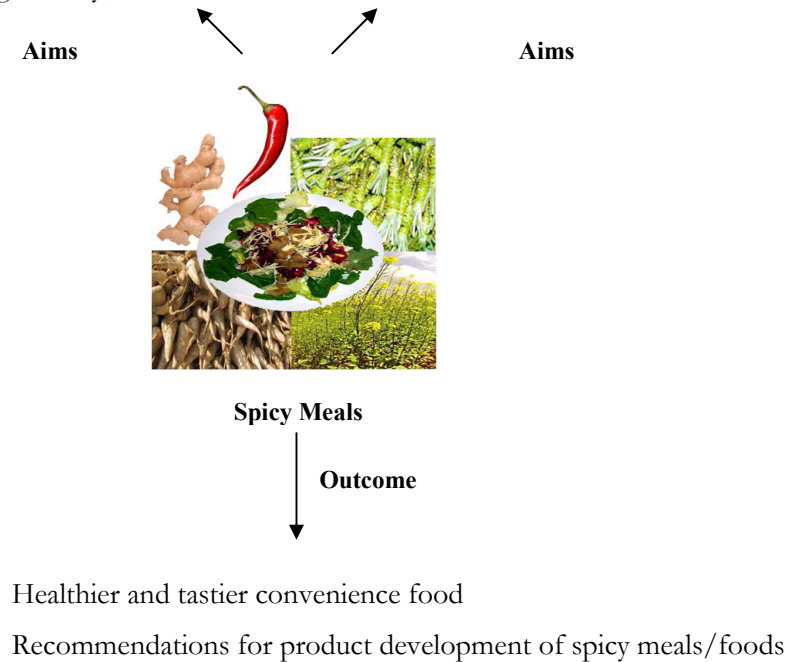


Figure 1.1

Obesity

During prolonged periods of positive energy balance, where energy intake is higher than energy expenditure, excess fat will be stored even though the body will try to compensate for the increased energy intake by increasing energy expenditure. The consequence of such a chronic imbalance can be development of obesity. The ability to store excess energy, especially fat, can be regarded as a survival mechanism evolved to ensure that humans store energy when food supplies are in excess to aid survival in times of starvation. Under normal or ideal conditions people have a stable weight where energy intake equals energy expenditure ¹.

According to the World Health Organization (WHO), body mass index (BMI) is used to classify people as normal weight (BMI: 18.5- 24.99 kg/m²), overweight (BMI ≥ 25 kg/m²), pre-obese (BMI: 25-29.99 kg/m²) and obese (BMI ≥ 30 kg/m²) ^{2,3}. Many attempts have been made to understand the main mechanisms for development of obesity and the general belief is that the common type of obesity is a consequence of complex interactions between genetics and environmental factors controlling eating behaviour and physical activity ^{4,5}. A change towards more high fat and high energy dense food as well as a more sedentary lifestyle with less physical activity due to transportation by car and more time spent in front of television and computers have been suggested as possible main causes of the rapid epidemic increase in obesity ⁶⁻¹⁰. Energy expenditure and substrate oxidation are some of the factors considered important for development of obesity. The sympathetic nervous system (SNS) has been identified as a regulator of energy expenditure and there is evidence that suggests that relatively low energy expenditure, acquired or genetically determined or low activity in SNS can be a risk factor for weight gain ¹¹⁻¹⁴ explaining why some people might be more likely to develop obesity than others. However since obese people generally have higher energy expenditure than lean persons ^{15,16}, obesity is unlikely mainly caused by a defect in energy expenditure. A chronic imbalance between fat intake and oxidation is also believed to increase the amount of adipose tissue and the risk of obesity ¹. Furthermore a low rate of fat oxidation, high respiratory quotient (RQ) as well as increased sensitivity towards insulin have also been associated with greater risk of gaining weight ¹⁷⁻²¹. Severe obesity has in rare cases been linked to single mutations in genes associated with regulation of eating behaviour and the leptin/melanocortin

pathway, which is essential for regulation of appetite and energy homeostasis ^{5,22-25}. Furthermore family studies have revealed that basic metabolic rate (BMR), height and weight are heritable traits ²⁶⁻³². Furthermore studies of genotypes have revealed that some people might be more successful in achieving weight loss or preventing weight regain than others ^{33,34} and the major challenge is to find more candidate genes or polymorphisms in these genes, that can explain genesis of obesity ^{35,36}. Neel et al. proposed in 'the thrifty-gene' hypothesis, that evolutionary pressure has put on genes favouring energy deposition suggesting that these thrifty genes might be activated by environmental factors such as sedentary life-style, food abundance and food composition to develop obesity ³⁷. The understanding of how obesity develops is however still very limited and deeper insight is needed to reveal how genetics, metabolic and physiological mechanisms interact with social and cultural behaviour.

The epidemic increase in obesity and its complications such as Type II diabetes mellitus, hypertension and heart diseases are major threats to the public health and the need for effective weight loss interventions is evident. Basically obesity can be treated by reducing energy intake or by increasing EE and commonly used weight loss approaches have therefore focused on restricting the diet and increasing physical activity. Physical activity is the most variable component of daily EE and can increase EE significantly ^{38,39} and its beneficial effect on maintaining weight loss and increasing fat oxidation support that physical activity can be an attractive strategy to prevent or treat obesity ^{6,40,41}. However, such behavioural strategies require a change in lifestyle, which might be difficult to maintain in the long term. Since thermogenesis and fat oxidation are mainly controlled by the SNS, approaches that affect activity of the SNS and its neurotransmitter norepineprine might offer a promising alternative for treatment of obesity ⁴²⁻⁴⁵. Weight loss approaches using herbal agents such as green tea or red pepper have recently gained more attention due to the beneficial effect on the targets in body weight regulation, satiety, metabolism and body composition caused by their ability to interfere with the activity of the SNS ⁴⁶⁻⁴⁸.

Appetite

By definition appetite is the selective desire to eat the food you are presented with whereas hunger is a non-selective desire to eat, which is related to the initiation of eating ⁴⁹. The subjective sensation of appetite is based upon complex integrations of various elements including desires for foods and drinks, eating pattern, which determines meal size and frequency and eating behaviour affecting food choices and preferences as well as day-to day variability in food intake. Appetite control can exert great influence on energy intake and thereby energy balance with risk of inducing excess energy intake leading to positive energy balance ⁵⁰. Thus appetite is of importance to regulation of body weight and a better understanding of appetite control can provide great insight into the genesis of obesity.

Blundell ⁵¹ explained appetite as a satiety cascade of events and processes which operate synchronously on three levels: 1) the psychological events controlled by hunger, satiety, perception, cravings and hedonic sensations which lead to energy and macronutrient intake during a meal or snack covering all aspects of eating behaviour, 2) the peripheral physiology and metabolic events and finally 3) neurotransmitter and metabolic interactions in the brain. The satiety mediated responses to food intake can be divided into the sensory, the cognitive, the pre-absorptive and the post-absorptive processes. During the sensory processes including the cephalic responses, the sensory properties of the meals including the taste, smell, texture, visual appearance, temperature and trigeminal stimuli initiate physiological responses in the mouth, such as excretion of saliva, and in the gastrointestinal (GI) tract that can motivate and anticipate food ingestion. Ghrelin, the hunger signal expressed in the gut and in the brain increase before a meal leading to increased sensation of hunger and ingestion of food. During the cognitive processes, prior experiences, learning and thoughts about the meal are used to make the decision that can lead to acceptance and ingestion. When nutrients are detected by chemo- and mechanoreceptors in the GI tract during the pre-absorptive phase, satiety signals such as PP, Amylin, Oleylethanolamide, CCK, GLP-1, GLP-2 and PYY are released. CCK is released postprandially, mainly in response to fat and protein content of the food whereas GLP-1 is stimulated by carbohydrates. In the post-absorptive processes the nutrients have been absorbed and released into the bloodstream where nutrient metabolites may be further metabolized in tissues or organs or may act as satiety signals together with other

endocrine signals. When food is sensed, ingested, digested and absorbed in the GI tract, satiety signals and sensory inputs provide negative feed-back via the vagus nerve to the nucleus tractus solitarius (NTS) in the brainstem and signals are then integrated in hypothalamus to limit meal size and inhibit further food intake ^{7,52}. Satiety is the complex of processes including the sequential release of satiety hormones that suppresses hunger within a meal and cause meal termination whereas satiation is the inhibition of hunger after food consumption that determines the length of the inter-meal interval. Satiation and satiety therefore act together to determine eating pattern ⁵³. Fullness is defined as a sensation of the degree of stomach filling and ‘prospective food consumption’ as an indicator of the supposed amount of forthcoming food intake.

While the satiety cascade involves mechanisms responsible for the short-term or episodic regulation of appetite that affects our eating behaviour, long-term regulation ensures energy homeostasis via regulators such as leptin, excreted from adipose tissue, and insulin. These long term regulators can activate taste receptor cells and mediate signals via the vagus nerve, which are integrated in several areas of the brain (hypothalamus, hippocampus, neocortex, thalamus, the brainstem) to inform about the energy state of the body and thereby maintain control of the overall regulation of appetite ^{7,54-59}. Leptin is thought to interact with NPY, a potent neurochemical substance important for appetite control, to be involved in the melanocortin system and several other neural mechanisms involving taste perception, food reward and hedonic response whereas insulin has been suggested as a body weight signal with capacity of influencing appetite control ⁷. Ghrelin is, however, both involved in episodic and long term regulation of energy intake, by initiating and suppressing hunger from meal to meal and over longer periods of time where ghrelin is affected by factors related to fat mass ⁵⁰.

The satiating effect of the macronutrients has been suggested to follow a hierarchy where protein is the most satiating followed by carbohydrate and finally fat ⁶⁰⁻⁶³. Accordingly, foods with varying macronutrient composition will correspond differently with the processes of the satiety cascade and will exert different effects on satiation and satiety. However, the short-term effects of fat and carbohydrate on appetite and energy intake remains controversial and it has been argued that such a satiating hierarchy might not exist and that the differential satiating effects

observed for the macronutrients are caused by differences in energy density of the test meals and study design ⁶⁴⁻⁶⁸.

Energy intake

WHO recommends that 55-75 % of total energy intake should come from carbohydrates (50-100 % of total body carbohydrate stores), 15-30 % of energy from fat (1 % of body fat stores) and 10-15 % of total energy intake from protein (1 % of protein stores) ⁶⁹.

Even though appetite is well regulated, many internal conditions (hunger, desires, physical activity, mood, memory, liking, physiological responses) as well as external factors (food culture, eating pattern, social interactions, health concerns, convenience, culinary context, and time used on eating) can influence and disturb the regulation of food intake.

It is generally accepted that palatability of food has a positive effect on food intake and meal size ⁷⁰. Furthermore palatable food has been found to increase hunger, prevent food compensation and shorten the inter-meal interval suggesting that palatability exert an effect on energy intake through modulation of appetite ⁷¹⁻⁷⁴. Not surprisingly some studies have shown that increased food intake as a consequence of increased palatability made subjects feel less hungry and more satiated ^{74,75}. Interestingly the presence of strong satiety does not down regulate palatability ⁷⁶. Since energy-dense food tends to be more palatable, learning about flavour – energy associations based on the post-ingestive consequences of consuming such foods is important to regulate energy consumption accordingly ^{73,77,78}. However the sensory inputs and post-ingestive consequences following ingestion of palatable food could undermine the cognitive processes of learned appetite for food with different energy density and thereby influence choice of foods as well as energy intake and lead to weight gain ⁷⁸. In naturalistic settings, palatability of the food was found to be positively related to larger meal sizes although palatability of the foods only explained minor variations in food intake and food choices ⁷⁹⁻⁸¹.

Several short term meal studies have revealed that energy intake is positively affected by greater variety, portion size and energy density of the foods whereas amount of food eaten is relatively consistent regardless of energy content ^{70,73,82-91}. Experiments with high volume, low energy dense soup have shown to increase satiation when compared with a low volume soup of same energy content, however without reducing energy intake at a subsequent meal suggesting that the effect of volume on energy intake rely both on amount consumed and energy density ⁸⁴. Over long term, energy intake is affected by energy density of the food through the composition of the macronutrients whereas energy density of drinks do not affect energy intake ^{87,92}. Furthermore, variation of energy density is compensated for by adapting portion size resulting in less variation in energy intake over the long term than during short term ⁸⁷. Memory plays an important role in developing such learned satieties that can control meal size. Thus enhancing memory for a recent meal has been found to suppress energy intake at a subsequent meal ⁶⁸.

Food cultures, that are characterized by its traditional dishes, basic ingredients and unique flavour principles, can also partly guide energy intake by its defined portions, food unit sizes, and meal frequencies ⁸². The recent increase in availability of convenience food has changed traditional eating patterns with tendencies towards larger portions sizes and more frequent snacking occasions in the US ^{82,93-95}. Contrary to these findings Danish people still seem to follow a structured eating pattern consisting of three main meals supplemented by a mid-afternoon and a late evening snack and main meals are still preferably eaten at work or at home with the family ⁹⁶.

Social interactions during a meal are also important for determining both what and how much people eat. Generally people tend to eat more when they eat in a social context, where family and friends exert the strongest social impact on energy intake ⁹⁷. Ingestion of a meal in a restaurant-like environment, with social interactions and food choices, revealed that combining these three contextual factors led to greater energy intake than when the food was consumed alone facing a wall ⁹⁸. However, some findings suggest that the greater food intake observed in the presence of social interactions and enhanced culinary context, might be caused by the increased meal duration due to the more favourable conditions ^{99,100}. Furthermore, a study manipulating social norms has shown that people eat more palatable food when

they believe other participants have eaten a great portion too, whereas less is eaten when they think that others only had a little amount of food ¹⁰¹.

Furthermore a high intake of carbohydrate, fat and protein in the morning reduced daily carbohydrate, fat and protein intake respectively, suggesting that morning intake is important for total intake of the day and that the reduced intake is macronutrient specific ¹⁰².

Liking and Desires

Eating can provide us with great feelings of pleasure or aversion that are crucial for the selection and consumption of food. Humans are born with a preference for sweet taste and innate liking for fat stimuli, which are believed to have been evolved to promote sufficient food intake of high energy dense foods ^{7,103,104}. Liking can be defined as a conscious immediate pleasure or positive affective state however liking has also been suggested to contain unconscious emotions ¹⁰⁵⁻¹⁰⁷. Desires, which are the intrinsic motivation to engage in eating a food, now or in the near future, can be driven by liking and is important for development of preference for specific foods ^{108,109}. The conscious desire to eat a specific food is developed from a complex integration of cues derived from the physiological state, expected pleasure from eating based on sensory memory and learned associations and finally external associations involving learned and cognitive behaviour ^{108,109}. Desires, liking and preferences are therefore all important for our food choices and food intake.

The hedonic processes and the reward, which follow food consumption, are mediated via the gustatory and olfactory sense. Sensory inputs are integrated in several areas of the brain involved in hedonic processing, possibly orbitofrontal cortex (OFC), nucleus accumbens, lateral hypothalamus, ventral pallidum and the brainstem, where complex interactions between brain neurotransmitters, including glutamate, opioids (endorphins) endocannabinoid and dopamine pathways and the associated receptor systems occur ¹⁰⁵. For some people the reward of a specific food can be so strong that actual cravings and food addiction develop. This addiction is believed to be related to sensitivity towards reward and the dopamine activity in the brain ^{110,111}. Deficits in dopaminergic systems have been suggested to

induce overeating in order to compensate for the poor food stimulation and to satisfy desires adequately.

The liking for spicy foods is most likely to be acquired in childhood after several exposures to oral burn ¹¹²⁻¹¹⁴. Preference for hot spices might develop simultaneously with liking for certain foods containing hot spices and other pleasant flavours, which thereby transforms the initial painful sensations into pleasurable piquancy ¹¹⁵. The novelty, the flavours and the surprising effects of the oral burn may cause arousal, which provides enjoyment at constrained risk for thrill seeking people, might also aid liking for spices ^{112-114,116}. Learning about beneficial effects of spices such as their ability to promote cooling (by sweating) and digestion, their antimicrobial effect and the fact that spices can be a good vitamin source might also explain why people accept hot spices ¹¹². Furthermore desensitization of oral burn might make the food less hot and explain why people can tolerate oral burn ¹¹⁶. Liking for spicy foods might also depend on sensitivity towards oral burn. Generally females are more sensitive to pain and might therefore find oral burn less tolerable ¹¹⁷. For people who are regular eaters of chilli, oral burn can be experienced as very pleasurable, whereas food without hot spices can be perceived bland and unappetizing ¹¹³. The fact that familiarization with chilli can enhance the liking of spicy foods indicates potentials for introducing more spices into food cultures not accustomed to hot spices. Chillies have even been suggested to cause addiction, however no evidence has proven that frequent use of chilli meet the criteria for an addiction ^{118,119}. However, when the oral burn from chillies is sensed by the trigeminal nerve and transmitted to the brain via the neurotransmitter substance P, the brain could plausibly react by releasing endorphins, the natural morphine drug, to relieve the pain from the oral burn ¹¹². The endorphin release could result in a kick or rush sensation, and learned associations between the pleasant emotional state and oral burn could explain why the liking for oral burn develops and may lead to an addiction-like state ¹¹².

Perception of a meal – An interaction between senses

The flavour rich experience we get from eating is a complex integration of inputs from the senses involved in perception of foods ¹²⁰. Vision is important to initiate eating since we use visual cues and previous experiences to judge and accept the food we are presented to. Already when we see palatable food, saliva is excreted to

aid the transport and digestion of food components in the mouth. The five basic tastes from the food are released by chewing and are transported via the saliva to the gustatory receptors for sweet, sour, bitter, salty and umami on taste cells, which are arranged in taste buds in circumvallate, fungiform and foliate papillae of the tongue. When tastes bind to the taste cells, primary gustatory neurons of the chorda tympani, glossopharyngeal and the vagus nerve are activated and impulses are projected to the NTS, where the signals are sent via thalamus to the primary taste cortex (frontal operculum and insula) ¹²¹⁻¹²³. Each taste cell contains the receptors for each of the basic tastes; however taste cells and the neurons in the taste cortex respond differently to different basic tastes and their concentrations. Furthermore taste as well as smell play a role in regulation of GI secretion ¹²⁰. The aroma compounds, which are released by chewing the food, reach the smell epithelia in the nostrils through the retronasal channel. The smell epithelium consists of the olfactory nerve cells, which are submitted in a mucus layer that facilitate the transport of aroma compounds to the nerve cells. A single aroma compound can bind to one or few of the nearly thousand different olfactory receptors on cilia of the nerve cells depending on the shape of the smell molecule. When the aroma compound binds to the smell neuron, a nerve impulse is emitted and transported to the olfactory bulb, a part of the primary smell area of the brain. The olfactory bulb nerve cells are organized in glomeruli according to their type of receptors and signals are transmitted by the first cranial nerve to the primary smell regions in the brain (piriform cortex, amygdala, periamygdala, entorhinal cortex) where the intensity of the smells are interpreted. The primary smell regions are closely connected to the limbic system (hypothalamus, hippocampus, amygdala) that deals with our unconsciousness, sexual instinct, feelings, motivation and memory. An appealing or disgusting smell can therefore provoke strong feelings and recall memories. The smell signal is then transferred to the secondary smell regions, mainly the OFC and insula, to identify the quality of the smell and to integrate smell with other sensory inputs. Volatile aroma compounds emitted from the food can also be inhaled through the nasal cavity (the orthonasal pathway) and reach the smell epithelium. Aroma compounds inhaled through the orthonasal pathway primarily act to motivate towards eating. The importance of smells to achieve the full eating experience is easily realized when the nasal cavity is blocked by a cold, which makes the food flavourless. The mouth feel of the food, such as the texture, the elasticity and the crunchiness of the food are evaluated by the sense of touch whereas audition can be important for perception of crispy foods. Apart

from the far senses (vision and audition) and the near senses (taste, smell and touch) another near sense, the trigeminal sense, stimulated by irritants, thermal and tactile stimuli evoking pain can also be essential for appreciation of many foods^{112,124,125}. Finally, interoception, which senses the physiological condition of the body, is of importance for the termination of eating. All sensory inputs from eating are integrated in the OFC to determine the sensory properties of the food, to recognize the food and finally to make hedonic responses to it, altogether qualities that enable us to gain the full eating experience^{7,126,127}. Neural activity in OFC is also regulated by hunger¹²⁸.

The sensory properties of foods play an important role in the way people select their food and how much they eat. The importance of sensory properties of the food for energy intake and food choices become evident with sensory-specific satiety (SSS), a phenomenon where the pleasantness ratings of a specific food drop relative to foods that have not been eaten¹²⁹. SSS is believed to arise from the sensory stimulation accompanying ingestion of food and might function to affect meal termination and to ensure that a variety of foods with different sensory properties and nutritional value are consumed^{70,130}. Since differences in taste and smell sensitivity have been suggested to affect both food choices and energy intake^{104,120,131-133}, decreased sensitivity towards taste and smell could explain why some people increase their energy intake and eat more energy dense food with risk of developing obesity.

The trigeminal sense

The trigeminal nerve (5th cranial nerve), which is a trigeminus nerve with free nerve endings going to the mouth, nose and eye region, senses pain evoked by irritants, thermal and tactile stimuli¹¹². Well-known irritants of the trigeminal nerve are capsaicin in chilli, allicin in garlic, diallyl sulfide in onion causing pain in the eyes and isothiocyanates in mustard, horseradish and wasabi causing tickling nose^{116,124}. Other common irritants are piperine from black pepper, cold and warm temperatures, carbon dioxide from soda, zingerones from ginger and cooling from menthol oil¹²⁴. Irritants such as capsaicin, piperine and zingerone, allicin, protons and heat, as well as N-arachidonoyl-dopamine (NADA) all bind to the transient receptor Potential Vanilloid receptor 1 (TRPV1), a nonselective cation channel

found on the free nerve ends of the trigeminal nerve in peripheral sensory fibres and in the brain ¹³⁴⁻¹⁴². Oral irritation occurs when the irritant diffuses across the epithelium where it binds to the TRPV1 on trigeminal nerve endings, which are in close contact with filiform papillae. When the TRPV1 is activated by irritants, the neurotransmitter substance P is released and afferent fibres project the signal via the lingual nerve to the brain stem trigeminal complex, particularly subnucleus caudalis (Vc), which contains neurons responsive to oral chemical irritants ^{112,143-149} (see figure 1.2). Henceforth the trigeminal neurons send information to the somatosensory thalamus and cortex ¹¹². Irritants such as acids and nicotine can however interact with other receptors, respectively the acid-sensing ion channel (ASIC) receptors and nAChRs receptors ^{112,150}.

Stimulation of the trigeminal sense can have a slow onset but when the burning or tickling sensations are in progression they can be very persistent and last several minutes after expectoration or swallowing of the irritant ^{116,125,151}. Unlike gustation and olfaction, the trigeminal sense does not adapt to continuous stimulation however sensitization can occur, which is observed as an enhanced sensory response caused by repeated stimulation of irritant applied with sufficiently short interstimulus intervals (ISI) ^{112,124,125,144,152}. The ISI is crucial to the sensory response since irritants can also provoke desensitization, a reduced sensory response, observed when capsaicin is reapplied after a sufficient rest period ^{112,125,144,152}. Stimulus-induced recovery (SIR), where the sensory response slowly enhances after desensitization, can occur when a repeated activation is first followed by a rest period and then a continuous activation ^{112,153}. Sensitization has been observed during repeated stimulation with capsaicin, piperine, citric acid and NaCl whereas desensitization has been observed after stimulation with capsaicin, piperine, zingerone, NaCl, nicotine, ethanol, cinnamaldehyde menthol, mustard oil and citric acid ^{112,141,144,154-158}. SIR has been observed after repeated capsaicin, menthol and citric acid application in both rats and humans ^{112,141,144,154-158}. Furthermore cross-sensitization between NaCl and capsaicin, piperine and zingerone as well as cross-desensitization between menthol and capsaicin, mustard oil and capsaicin, nicotine and capsaicin, has been observed ^{112,155,156}.

Hot spices and their effects on sensory properties of the food

In all food cultures unique flavour principles have been important in preparation of traditional meals. The flavours do not only provide nutritional value but enhance the pleasure of eating by making the meal more exciting, balanced and complete. In modern cooking traditional foods can be prepared in new and exiting ways with an exotic twist of flavours from all over the world that can provide us with greater food variety and pleasure.

Hot spices such as chilli peppers, black pepper, ginger, horseradish, wasabi and mustard add the burning and tickling sensations and might furthermore interact with tastes and flavours to enhance the overall flavours of food ¹²⁴. Generally, irritants produce tastes and odours at low concentrations and trigger pungency at higher concentrations with few exceptions such as the taste and odourless carbon dioxide ^{116,124,125,159-163}. Furthermore, irritants have been proposed to enhance the flavour intensity of foods however, so far, no clear evidence exists showing that capsaicin increases flavour intensity ^{125,164,165}.

Studies exploring how the trigeminal sense interacts with the gustatory and olfactory senses have mainly been conducted with capsaicin. Both the persistence and the intensity of the oral burn from chilli are affected by the capsaicin concentration, the profile of the capsaicinoids in the chilli product, the serving temperature, the ISI, the food matrix, individual sensitivity and prior experiences with oral burn ^{112,124,132,144,151,154,156,166-169}. Human studies have confirmed that capsaicin has suppressive effect on gustatory sensations ^{124,170-172}. However, the reported effects on taste qualities vary, showing only a general consensus, that capsaicin reduces perceived sweet intensity in both solutions and in foods ^{124,151,171,172}. Oral irritation from capsaicin has also been found to suppress olfactory sensations, whereas the influence of capsaicin with complex flavours is less clear ^{151,170,171}. Furthermore some studies have shown that desensitization by capsaicin decreased taste intensity whereas others did not find any effect of capsaicin desensitization on taste ^{172,173}. Few studies have looked at how tastes, flavours and irritants interact in complex foods. Water-based samples with capsaicin are generally perceived as more intense than equally spiced food or liquids with more complexity such as cheese, starch and oil-based carriers ^{167,174}. Increasing fat levels of food (starch paste and cheese sauce) have also resulted in lower oral burn scores

^{167,175}. Contrary to these findings increasing fat levels in chilli spiced chicken patties increased oral burn ¹⁷⁶, which suggests that the effect of fat concentration on oral burn is dependent on the food matrix. Furthermore red hot pepper increased the perceived oiliness of high carbohydrate (HC) meals to levels comparable to high fat (HF) meals, which indicates the potentials of adding red pepper to low fat meals to increase the perception of fat ¹⁷⁷. Additionally chilli pepper has been shown to reduced intensity of warmed-over flavour (WOF) in chicken patties, suggesting that the antioxidant properties of chilli pepper might be useful to improve the culinary quality of precooked meat products ¹⁷⁶. Prescott et al showed that desensitization occurred during consumption of hot soup and chilli con carne whereas no evidence for sensitization was found during food consumption ¹⁷⁸. It was therefore suggested that sensitization might be prevented as a result of the complexity and the fat content of the food.

Even though research of the trigeminal sense has focused on investigating if and how trigeminal stimulation from irritants interact with gustatory and olfactory sensations, no clear mechanism has yet been revealed. Capsaicin suppression of taste and flavours could be a psychological, cognitive phenomenon, where the dominating oral burn draws attention away from other sensations ^{172,179}. Several exposures of chilli could make the oral burn seem less dominating, explaining why frequent users of chilli find the oral burn less dominating than infrequent users of chilli ^{170,171}. Another possibility is that suppression of taste by oral burn might produce desensitization in taste or flavour perception ^{163,173} suggesting that frequent users could be in a chronic state of desensitization due to the regular chilli consumption ^{112,125,171,173}. Taste suppression could also be caused by interactions between trigeminal, gustatory and olfactory sensations in NTS, thalamus or cortex ^{171,172}. Most theories suggest that interactions between gustatory, olfactory and trigeminal stimuli occur via central neural integration of sensory input and/or via peripheral effects ^{124,170-173,179-181}.

Metabolic and appetite regulating effects of hot spices, green tea and sweet peppers

Red peppers, genus *Capsicum*, belong to the plant group of the Solanaceae family and are one of the oldest cultivated crops in America ^{182,183}. The five most cultivated species of genus *Capsicum* (C) include green and red peppers ranging

from the non-pungent peppers of the *C. annuum* L. (big sweet peppers) and *C. baccatum* L. species to the very hot red peppers including *C. baccatum* L., *C. pubescens* R. and P., the *C. frutescens* L. (known from Tabasco) and finally the *C. chinense* Jacq, where the Habanero (Scotch Bonnet) and Bhut Jolokia are known to be the hottest chillies in the world ¹⁸⁴.

The oral burn from hot peppers is caused by a group of ten alkaloid compounds collectively called capsaicinoids. The capsaicinoids are produced in the glands of the placenta, which are the white strings in the pepper, which holds the seeds in place. The seeds are bitter and do not contain capsaicinoids but can absorb the hot compounds and thereby be perceived as burning ¹⁸². The strength of the hot peppers is determined by genetic factors, weather, growth conditions and the age of the pepper. Generally, environmental stress will enhance the concentration of capsaicinoids, that typically range from 100 µg - 2.5 mg capsaicinoids/g hot pepper ¹⁸⁵⁻¹⁸⁷. The capsaicinoids consist of an aromatic part, vanillylamine, linked with an acyl residue, consisting of C₈ -C₁₃ fatty acids ¹⁸⁸. The two major capsaicinoids, capsaicin and dihydrocapsaicin, are believed to be the main contributors to the oral burn whereas norcapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nornordihydrocapsaicin and nornorcapsaicin (homocapsaicin/homodihydrocapsaicin) only occur as minor components ¹⁸⁸⁻¹⁹⁰. The Scoville heat test, which was originally used to rate the pungency of chilli peppers, is based on the principle that chilli pepper extract is diluted in sugar water until no heat is detectable by a sensory panel; the dilution-fold is reported as Scoville heat units (SHU). Nowadays pungency can be rated in ASTA pungency units, which correspond to the capsaicinoids concentration in the chilli pepper usually determined by high performance liquid chromatography (HPLC). ASTA pungency can be converted to SHU by multiplying by a factor of 15.

Capsaicin has in several short-term indirect calorimetric studies shown to increase EE ^{177,191-194} (table 1.1). A chilli and mustard sauce increased diet induced thermogenesis (DIT) in men by 29 % immediately after ingestion whereas an overall increase in metabolic rate of 25 % was observed 150 min. after ingestion of the spicy meal when compared to a non-spicy control meal ¹⁹². Similarly Yoshioka et al. found a 23 % increase in EE at rest in males immediately after consuming a HC breakfast with chilli when compared to the control meal ¹⁹⁴. Chilli pepper has furthermore shown to promote carbohydrate oxidation in males, fat oxidation in

females as well as to stimulate the release of triacylglycerol (TG) during exercise in males ^{177,193,194}. Greater effects on DIT and lipid oxidation were however found when chilli peppers were added to a HF meal when compared to HC meal ¹⁷⁷. Inconsistent results on the effect of red pepper on substrate oxidation and EE could be caused by the study designs, which differ in chilli products and capsaicin concentrations, genders as well as the energy balance conditions that were used ^{46,177}. Stimulation of EE by chilli pepper was inhibited by β -blockers and capsaicin was found to increase the release of catecholamine's, suggesting that capsaicin increases the SNS through adrenergic stimulation ^{177,194}, which then promotes EE and substrate oxidation in humans. The metabolic effects of capsaicin have also been studied in animals. These studies suggest that capsaicin is absorbed through the GI, transported into the blood stream with serum albumin, where it stimulates the visceral afferent sensory neurons, the spinal neurons and finally the adrenal sympathetic nerves are activated due to enhanced adrenal catecholamine secretion. Catecholamine's react with β -adrenergic receptors in liver and adipocytes enhancing glycogenolysis and lipolysis, which results in formation of energy-producing substrates that are circulated throughout the body and converted into heat in peripheral tissues such as muscles ¹⁹⁵⁻¹⁹⁷ (figure 1.2).

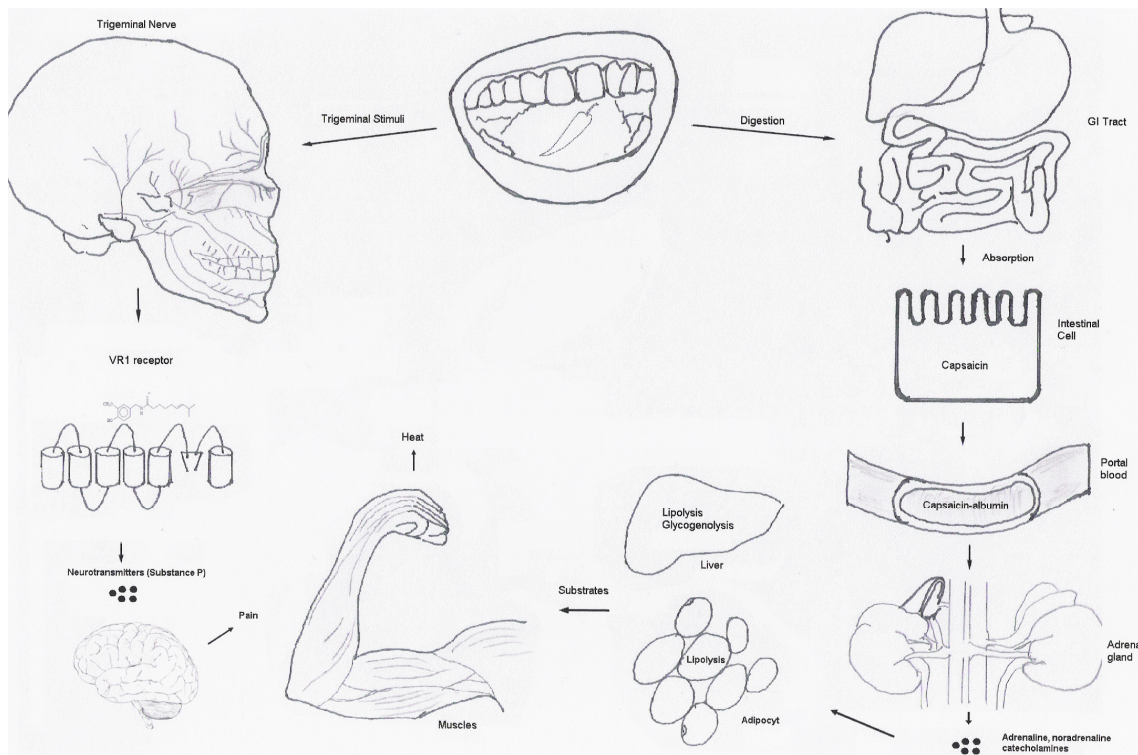


Figure 1.2 Oral and gastrointestinal effects of capsaicin ingestion. Oral pathway (left): capsaicin activates the trigeminal nerve through binding to its TRPV1 receptor, substance P is then released from trigeminal nerves and nerve signals inform the brain about the pain stimulation. GI pathway (right): After ingestion, capsaicin is absorbed in gut cells, released into the blood, transported via albumin to the adrenal gland, where capsaicin is believed to stimulate catecholamine release, which activates lipolysis in adipocyt and in the liver, as well as glycogenolysis in the liver, thereby enhancing substrate oxidation in the muscles, which can use the excess energy to produce heat ¹⁹⁵⁻¹⁹⁷.

Traditionally chilli peppers are used to stimulate appetite in humans during the hot and humid summers in Japan ¹⁹⁸. In contrary to these findings scientific studies have revealed that chilli pepper ingested with breakfast increased satiety and reduced energy intake at lunch by reducing fat intake as well as protein intake in females whereas chilli pepper given with an appetizer decrease energy and carbohydrate intake at lunch in men ¹⁹⁸ (see table 1.2). The effects on satiety and reduction in protein and fat intake were larger with HF meals compared to HC meals ¹⁹⁸. No effects on energy intake were however found using moderate doses of chilli pepper whereas high doses increased satiety, suppressed hunger and gave largest reduction in energy and fat intake when the chilli pepper was administered orally compared to GI stimulation ^{199,200}. A change in daily macronutrient intake was also observed since red pepper given orally or in capsules induced an increase in carbohydrate intake and decrease in fat intake ²⁰⁰. Furthermore increased activity

of the SNS after ingestion of red pepper indicates that the reduction in energy intake could be due to the anorectic effect of catecholamine's ^{46,198}. These findings suggest that sensory stimulation and chilli concentration are of importance to the total response ^{199,200} and indicate promising short term effects of chilli peppers on energy expenditure, energy intake and appetite.

The literature on the long term effects of chilli pepper is scarce. However Lejeune et al. ²⁰¹ performed a long-term study on moderate overweight subject, who after a modest weight loss followed a maintenance intervention with continuous capsaicin administration (table 1.3). After three months, capsaicin still increased fat oxidation and resting EE, however these effects were too small to limit regain of body weight ²⁰¹. Long term administration of capsaicin had no effect on hunger suggesting that effects on appetite might be a short term effect or that sensory stimulation from oral burn is needed to suppress appetite ^{200,201}. Due to burning sensations in the stomach ten subjects had to take half a dose of capsaicin. Other studies found no beneficial effects of long-term administration of chilli on BMR and several metabolic parameters ²⁰²⁻²⁰⁴. A chilli spiced meal however lowered insulin release after four weeks of respectively a bland and a spicy diet when compared to non-spicy conditions ²⁰².

Table 1.1 Short-term studies on the effect of capsaicin on energy expenditure.

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Henry and Emery ¹⁹²		Meal Treatment \pm 3 g Chilli, 3 g mustard sauce	Mustard and chilli increased EE 29 % 0-15 min 25 % over 150 min.	Red pepper enhanced DIT
Yoshioka M. et al. ¹⁹⁴	8 males	Meal breakfast HC: P:F:CHO = 15:25:60 Treatment \pm 10 g Chilli (30 mg capsaicin) Condition rest	Chilli increased EE by 23 % 0-30 min. Lipid oxidation 0-30 min. RQ 50-100 min. CHO oxidation 50-150 min. β -blockers inhibited the red pepper induced increase in EE.	The effect of red pepper on EE is mediated via β -adrenergic stimulation
Lim K. et al. ¹⁹³	8 males	Meal breakfast HC: P:F:CHO = 15:25:60 Treatment \pm 10 g Chilli (30 mg capsaicin) Condition rest or exercise	Chilli increased (during rest and exercise) RQ 60-90 min., Blood lactate 60-150 min. Epinephrine by 81 % Norepinephrine by 76% 0-30 min. during Chilli increased (during exercise) Triacylglycerol 120-270 min. Non-significant increase in EE (10 %).	Chilli increases SNS and promotes CHO oxidation and release of TG as energy fuel.
Yoshioka M. et al. ¹⁷⁷	13 Japanese females	Meal Breakfast HC: P:F:CHO =15:25:60 HF: P:F:CHO =15:45:40 Treatment \pm 10 g Chilli (30 mg capsaicin) Condition rest	Chilli increased DIT, lipid oxidation over 210 min. (Effects HF> HC) Oiliness (Effects HC >HF) Chilli decreased CHO oxidation (effects HF> HC), palatability, taste, RQ 30-60 min.	Chilli increased SNS. The different effect of chilli on substrate oxidation could be due to gender differences, since women have more type I fibres with β -adrenergic receptors in their skeletal muscles than men and thereby might get more β -adrenergic stimulation from chilli peppers.
Chaiyata P. et al. ¹⁹¹	10 Thai females	Meal Glucose drink Treatment \pm 5 g Chilli Condition rest	Chilli increased metabolic rate 5-20 % 5-30 min. Chilli decreased plasma glucose by 20.6%.	

CHO, carbohydrate; DIT, Diet-induced thermogenesis; EE, Energy expenditure; ; F, fat; HC, high carbohydrate; HF, high fat; min., minute(s), P, protein; RQ, respiratory quotient; SNS, sympathetic nervous system; TG, triacylglycerol; Time 0 min. = meal termination, Time in min. shows when the effect was apparent.

Table 1.2 Short-term studies on the effect of capsaicin on energy intake and appetite.

Study	Subjects	Study design	Effects on Energy intake and appetite	Mechanisms/conclusions
Yoshioka M. et al ¹⁹⁸	Study 1 13 Japanese females	Meal breakfast and ad lib lunch HC: P:F:CHO =15:25:60 HF: P:F:CHO =15:45:40 Treatment \pm 10 g Chilli (30 mg capsaicin) Condition rest	Chilli reduced Appetite: Desire to eat, Prospective food consumption, hunger before lunch (Effect HC> HF), Energy intake at lunch in women : Protein: 20 % (HF), 6 % (HC) Fat intake: 17 % (HF), 11 % (HC) Energy intake at lunch in men : Energy 11 %, CHO 18 % Chilli increased SNS:PNS activity ratio in men	The decreased food intake could be due to increased release of catecholamine's (increased SNS), which might have anorectic effect.
	Study 2 10 Caucasian males	Meal Appetizer + ad lib lunch Treatment \pm 6 g Chilli (18 mg capsaicin) Condition rest		
Yoshioka M. et al ¹⁹⁹	16 Japanese males	Meal soup and ad lib lunch Treatment \pm 0.064 g red pepper (0.192 mg capsaicin, Medium hot) and 0.923 g red pepper (2.769 mg capsaicin, maximum tolerable dose) given in soup or capsules with soup Condition rest	Chilli reduced Fat intake by 15.5 % in hot soup at maximum tolerable dose of red pepper reduced (Effect hot soup> capsules) SNS:PNS at the hottest dose (too spicy). Chilli increased SNS:PNS from low to maximum tolerable dose	High Chilli doses necessary to suppress energy intake. Increase in SNS not dose dependent. Main site of action stomach and/or small intestine whereas the mouth is less important. Japanese male and female respond similar to red pepper, by reducing fat intake, this is different from Caucasians. (Japanese diet is low in fat)
Westerterp- Plantenga et al. ²⁰⁰	12 men 12 women Caucasian	Meal breakfast, lunch, snacks, dinner Treatment \pm preload of 0.9 g red pepper (2.25 mg capsaicin) served in juice (orally) or capsules (GI) 30 min. before every meal Condition daily living	Capsaicin increased CHO intake and satiety Capsaicin reduced Daily energy intake by 10 and 16 % (Capsules, orally) after lunch and dinner, Fat intake, Hunger	Neurons in the OFC can be specifically tuned to capsaicin. Sensory perception of capsaicin reduced energy intake even further than the capsules (GI) Effect was related to perceived spiciness and change in food choice.

CHO, carbohydrate; GI, gastrointestinal; HC, high carbohydrate; HF, high fat; P, protein; F, fat; min, minute(s); OFC, orbitofrontal cortex; SNS, sympathetic nervous system; PNS, parasympathetic nervous system .

Table 1.3 Long-term studies on the effect of capsaicin on metabolism

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Lejeune et al. ²⁰¹	91 moderate overweight men and women BMI 25-35	Meal 4 weeks VLED (weight loss 5-10%) 3 months WM Treatment \pm 135 mg capsaicin in capsules/day for 3 months	Capsaicin Increased Fat oxidation (smaller RQ increase) Capsaicin decreased Insulin, leptin, Glucose, TG No effect Weight regain, energy intake, hunger scores EE, FFM	Substrate oxidation was affected by capsaicin on the long term Habituation to capsaicin? Lack of compliance
Ahuja et al. ²⁰²⁻²⁰⁴	36 subjects 22 women 14 men	Meal 4 wk. of bland diet (chilli-free) 4 wk. and chilli-diet Treatment \pm 30 g cayenne/day for 4 wk.	Chilli increased Heart rate in men Chilli decreased Insulin Augmentation index (arterial stiffness) No effect Glucose, lipids, lipoproteins, insulin, BMR, BP	No beneficial effects of chilli on several metabolic parameters. Chilli may attenuate postprandial hyperinsulinemia.

BMR, basal metabolic rate; BP, blood pressure; EE, Energy expenditure; FFM, fat free mass, RQ, respiratory quotient; TG, triacylglycerol; VLED, very low energy diet; wk, week(s); WM, weight maintenance

Even though compliance was not diminished because of these side effects a weight loss strategy using hot pepper may however be difficult to complete due to the strong oral burn and burning sensations in the stomach. Therefore the non-pungent red peppers could be an attractive alternative to hot peppers. The non-pungent red peppers contain either no or very low amounts of capsaicinoids. However, a group of structurally similar compounds, the capsinoids, has been identified in the nonpungent CH-19 sweet pepper (*Capsicum annuum* L.). The three major capsinoids, capsiate, dihydrocapsiate and nordihydrocapsiate are found in the ratio 5:3:1 and have the same acyl residues as capsaicin, dihydrocapsaicin and nordihydrocapsaicin whereas the aromatic part differs as capsinoid has a vanillyl alcohol instead of a vanillylamine^{188,205}. None of the capsinoids had pungency upon oral tasting¹⁸⁸.

Capsiate, the predominant capsinoid in CH-19 sweet pepper, has been shown to increase body temperature, oxygen consumption and adrenalin concentration as well as to promote carbohydrate and fat oxidation in mice to similar extent as capsaicin²⁰⁶⁻²⁰⁸. Significantly, lower level of serum TG and higher level of serum free fatty acids (FFA) and glucose were observed after capsiate administration, which further confirmed that adrenalin promotes substrate oxidation by enhancing lipogenesis in the liver and adipocytes as well as the glycogenolysis in the liver²⁰⁶. However changes in body temperature were different after administration of capsiate compared to capsaicin as was the time-lag in the concentration of adrenalin and oxygen consumption, which could be due to different absorption processes of the two compounds in the GI tract^{206,207}. In humans a single dose of CH-19 sweet pepper increased body temperature and oxygen consumption²⁰⁹ (table 1.4). Furthermore CH-19 sweet pepper increased thermogenesis and SNS activity in humans, to a similar extent as hot peppers²¹⁰. However, heat losses from neck and forehead were greater after hot pepper consumption than after CH-19 sweet pepper²¹⁰. Unlike hot peppers, CH-19 did not affect systolic blood pressure (BP) or heart rate (HR) suggesting that pungency and unknown mechanisms, other than SNS activity, might affect heat loss, BP and HR²¹⁰. In a recent study, repeated CH-19 sweet pepper intake for two weeks reduced body weight and enhanced fat oxidation by increasing the SNS, while no effects were seen on EE²¹¹. Thus CH-19 sweet pepper seems to promote EE in the short term whereas continuous administration of capsiate suppresses fat accumulation, however, less is known about how CH-19 sweet peppers affect energy intake and appetite.

Table 1. 4 Effect of CH-19 sweet pepper on metabolism

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Ohnuki et al 209	7 men 4 females Japanese	Meal No Treatment 0.1 g CH-19/ California-Wander per kg BW Condition rest , Indirect calorimetry, Infrared and electronic thermometer	CH-19 sweet pepper increased Body temperature 10-60 min. (Forehead 20 min., surface and wrist 10-15 min., neck 10-60 min.) Oxygen consumption 40 min. No effect RQ	Effect on oxygen consumption but not RQ suggest no effect substrate oxidation but effect on EE. Same increase in oxygen consumption as hot peppers. Increased forehead and wrist temperature suggest that CH-19 stimulates vasodilation in humans.
Kawabata et al 211	7 males :CH-19 sweet pepper group 5 males: control group Japanese	Meal standardized diet (1 wk adaptation, 2 wk. test) to keep BW stable. Treatment 0.4 g CH-19/kg body weight, split on 3 meals, two weeks administration.	CH-19 sweet pepper decreased Body weight, BMI fat mass, FFM, RQ, Total fat area No effect Energy intake/day, respiratory gas, oxygen consumptions, CHO oxidation, BP	Weight loss was significantly correlated with activity of SNS suggesting that reduced body weight and body fat accumulation are mediated by SNS.
Hachiya et al 210	5 males 7 females Japanese	Meal No Treatment 0.1g CH-19/california wonder per kg BW	CH-19 sweet increased SNS (LFC) and tympanic temperature similar to capsaicin. Forehead temperature and neck to smaller extent than capsaicin No effect Systolic BP, HR, HFC – unlike capsaicin	Heat loss greater after hot peppers than after CH-19 sweet pepper. CH-19 sweet pepper increases thermogenesis (tympanic temperature). No effects of CH-19 sweet pepper on HR, BP suggest that pungency and effects other than SNS might cause changes in BP, HR and heat loss in hot peppers.

BP, blood pressure; BW, body weight; CHO, carbohydrates; EE, energy expenditure; FFM, fat free mass; HFC, high-frequency component; HR, heart rate; LFC, low-frequency component; min., minute(s); RQ, respiratory quotient; SNS, sympathetic nervous system; Time: 0 min., treatment termination; Time in min. shows when the effect was apparent; wk., week(s);

Catechins and caffeine, two groups of active compounds in green tea, contribute to the beneficial metabolic effects of green tea observed in animal and human studies ^{44,212-222}. On short term green tea has been found to increase thermogenesis and fat oxidation in lean to mildly obese men following a weight-maintenance diet ⁴⁴ (table 1.5).

Two weeks administration of green tea was found to reduce body fat, cholesterol levels and blood pressure in Japanese normal to overweight subjects without further lifestyle changes ²¹⁷ (table 1.6). Long term administration of green tea with the usual diet was found to stimulate EE and to reduce body weight whereas green tea consumed with a diet of 90 % of the individual energy need reduced body fat and weight in normal to obese subjects ^{212,216} (table 1.7). Contrary to these findings, long term use of green tea after weight loss did not improve weight-maintenance and did not prevent weight gain in humans, however green tea reduced body weight, body fat and stimulated resting energy expenditure (REE) in low caffeine users ^{215,218}. When green tea was added to a long term low energy diet (LED), no anti-obesity effects were observed in overweight women however green tea was found to increase hunger ²¹³. These findings suggest that long term administration of green tea may be beneficial for prevention or improvement of obesity, by enhancing lipid metabolism. Few studies have looked at the effect of green tea on energy intake and appetite however Kao et al ²¹⁴ showed that epigallocatechin-3-gallate (EGCG) from green tea reduced food intake and body weight in rats within 7 days and that the effect of EGCG was dose-dependent.

Table 1.5 Short-term effects of green tea on metabolism

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Dulloo et al 44	10 men, young, healthy, lean to mildly obese	Meal WM diet 5-6 wk. P:F:CHO = 13:40:47 Treatment Green tea extract (50 mg caffeine + 90 mg EGCG), caffeine (50 mg) and placebo given at breakfast lunch and dinner. Respiration chamber indirect calorimetry	Green tea extract increased 24 hr EE by 3.5 % 24 hr norpinephrine in urine by 40 %, Greent tea extract decreased 24 hr RQ Caffeine increased 24 hr EE by 2.8 % No effect N in urine, HR	Green tea was more effective than the equivalent amounts of caffeine in stimulating EE. Green tea extract has thermogenic properties and promotes fat oxidation. May control body composition via SNS activation of thermogenesis

CHO, carbohydrate; EE, energy expenditure; EGCG, epigallocatechin-3-gallate; F, fat; HR, heart rate; N, nitrogen; P, protein; RQ, respiratory quotient; SNS, sympathetic nervous system; wk., week(s); WM, weight maintenance;

Table 1.6 Long-term effects of green tea on metabolism

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Westerterp-Plantenga et al ²¹⁸	76 males and females BMI 25-35 kg/m ²	Meal LED for 4 wk., 3 month usual diet (WM) Treatment green tea (270 mg EGCG + 150 mg caffeine per day) or placebo Condition 4 wk. LED and free living	Green tea reduced Body weight, waist, RQ, body fat during WM in low caffeine users. Green tea increased REE during WM in low caffeine users No effect of green tea-caffeine in High caffeine users during WM	Green tea improved weight maintenance in low caffeine users through stimulation of thermogenesis and fat oxidation.
Diepvens et al ²²³	46 females BMI 25-31 kg/m ²	Meal 1-3 day: energy balance 4-87 day: LED Treatment 1206.7 mg tea catechins + 236.7 mg caffeine/day Condition LED for 83 days	No effect of green tea on Blood parameters during weight loss	No benefits of green tea on blood when used as a part of a meal-replacement diet plan.
Nagao et al ²¹⁷	240 Japanese males and females Control group = 123 Catechin groups = 117 BMI 24-30 kg/m ²	Meal 2 wk. adaptation, 12 weeks usual diet Treatment 340 ml green tea (583 mg catechin) or control (96 mg catechin) per day	Green tea decreased BW, BMI, body fat ratio, body fat mass, waist circumference, hip circumference, visceral fat area, subcutaneous fat area, BP (only in subjects with BP > 130 mm Hg), LDL cholesterol	Reduced body fat may be related to the increase of EE. Decrease in BP and LDL cholesterol may be the result of increased body fat oxidation suggesting that green tea contributes to a decrease in obesity and cardiovascular disease risks.
Chantre et al. ²¹²	7 males 63 females BMI 25-32 kg/m ²	Meal usual diet Treatment 375 mg catechins (270mg EGCG) per day ingested morning and midday Condition free living, BW every 4 wk until wk 12	Green tea extract decrease BW by 4.6 %, WC by 4.48% Green tea extract Increase EE No effect cholesterol, BP, HR In vitro: inhibit gastric and pancreatic lipases, stimulate thermogenesis.	Increased thermogenesis mediated by a reduction in enzymatic degradation of NA and prolongation of action of sympathetically released NA. Adverse events such as abdominal pain, diarrhoea and increase of transaminases were reported.

BP, blood pressure; BW, body weight; EE, energy expenditure; EGCG, epigallocatechin-3-gallate; LDL, low-density lipoprotein; LED, low energy diet; REE, resting energy expenditure; RQ, respiratory quotient; wk, week(s); WM, weight maintenance;

Table 1.7 Long-term effects of green tea on metabolism (continued)

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Kovacs et al. 215	Green tea group: 36 females, 15 males Placebo group: 42 females, 11 males BMI 25- 35 kg/m ²	Meal 4 wk. of VLED,13 wk. of usual diet Treatment green tea (caffeine 104 mg/day, 573 mg catechins) or placebo for 13 wk. Condition 4 wk. of VLED,13 wk. WM	No effect of green tea on WM after body-weight loss Effect of green tea on WM with high habitual caffeine consumption. Higher regain with high habitual caffeine consumption	
Diepvens et al. 213	46 females BMI 25-31 kg/m ²	Meal 1-3 day: energy balance 4-87 day: LED Treatment 1125 mg tea catechins + 225 mg caffeine/day Condition LED for 83 days, measure EE on day 4 and 32	Green tea increased Hunger over time, prospective food consumption Green tea decreased thirst No effect weight, BMI waist:hip ratio, FM, FFM	No benefits of green tea for body weight or body composition when used as a part of a meal-replacement diet plan.
Nagao et al. 216	38 normal to overweight males	Meal 90 % of daily energy need Treatment 340 ml control beverage (21.8 mg catechin), green tea beverage (689.9mg catechin) consumed at supper for 12 weeks Condition Free living	Green tea reduced body weight by 1.5 %, BMI by 1.5 % WC by 2 %, skin fold thickness by 6.9 %, body fat mass by 3.7 5%, total fat area by 7.9 %, subcutaneous fat area by 7.5 %, (MDA)-LDL,	Catechins not only promote EE but also mildly reduced body fat in humans. Long-term consumption of green tea inhibits the formation of oxidized lipids (MDA-LDL), a risk factor for development of arteriosclerosis.
Ohmori et al. 219	22 normal weight males	Meal usual Treatment 7 cups of water (control) or 7 cups of green tea (542.5 mg catechins, 108.5 mg caffeine, 56.7 mg of vitamin c) per day for two weeks Condition free living	Green tea decreased MDA-LDL and ratio of MDA-LDL/LDL-cholesterol No effect of green tea on plasma LDL-cholesterol concentration	Green tea consumption may inhibit LDL oxidation In vivo. Daily consumption of green tea decreased serum MDA-LDL concentration but had no effect on platelet aggregation, platelet TX production, plasma and MMPs concentration.

BP, blood pressure; BW, body weight; EE, energy expenditure; EGCG, epigallocatechin-3-gallate; FFM, fat free mass; FM, fat mass; HR, heart rate; LDL, low-density lipoprotein; LED, low energy diet; MDA, malondialdehydmodified; NA, noradrenaline; VLED, very low energy diet; wk, week; WC, waist circumference; WM, weight maintenance;

Outline of the thesis

Part I, 'Sensory Properties of Spicy Meals' focuses on how trigeminal stimuli affect the sensory properties of food. Two articles investigate how oral burn interacts with respectively meat flavour and texture (paper I) and heat perception (paper II) in chilli spiced pork patties. Next a study (manuscript I) was conducted to reveal if interaction between taste and the trigeminal sense could explain observed suppression of tastes by capsaicin. The activity in sweet taste signalling pathways in rat taste cells were visualized by immunostaining ERK2 proteins on confocal laser scanning micrographs after stimulating rat taste cells with respectively sucrose or capsaicin and sucrose.

In part II of the thesis, 'Spicy Meals, Appetite and Energy intake', the effects of hot spices were assessed to investigate how hot spices affect appetite and energy intake. A meal study was performed to reveal if hot spices such as chilli pepper, horseradish, ginger, mustard and wasabi served with a meal could reduce energy intake and appetite at a succeeding ad libitum buffet (paper III). However we speculated that the effects of bioactive ingredients from hot spices and green tea might depend on energy balance and another meal study was conducted to investigate the effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake when added to the daily meals during respectively three weeks of negative and three weeks of positive energy balance (paper IV).

Methods

In part I ‘Sensory Properties of Spicy Meals’ it was investigated how texture and heat perception affect oral burn (paper I and II) as well as how oral burn affects the meat flavour of chilli spiced pork patties (paper I). The attributes oral burn and meat flavor were scored by a trained sensory panel using Time-Intensity (TI) methodology. TI was chosen over more static sensory methods, since TI is a dynamic sensory method with high resolution and validity regarding perception of food that allows persistent sensations such as oral burn to be tracked as it changes over time ^{224,225}.

From the TI curves of oral burn and meat flavour several parameters such as the slope of the increasing and decreasing phase, the plateau, area under the curve, maximum intensity, rate, duration and curve shape can be derived and analyzed by classical statistical approaches. Analysis of TI parameters can be effective in differentiating the samples and provide extra information not present in conventional data ²²⁶, however, curve properties may differ between stimuli and the parameters best describing the samples may differ from experiment to experiment ²²⁴.

Alternatively the raw TI curve can be analysed, which might implicate challenges to the data analysis due to a high number of data points, individual differences and difficulties in separate the sample variation from the variation due to individual differences between assessors ^{224,227}. Multivariate data analysis ^{228,229} can, however, provide global information on all aspects contemporaneously (assessors, samples and time profiles) and has previous been successfully applied on TI data ²²⁷. With Principal Component Analysis (PCA), the principal curve represents a weighted average of the individual curves, which gives a better representation of the individual curves than an average curve ²²⁴ and makes it possible to investigate if and how assessors differ. As TI curves are three-dimensional data (samples times assessors times TI profiles), feature-extractions or similar methods have to be used in order to model these data with PCA. On the other hand, multiway models (such as PARAFAC and PARAFAC2) allow handling of multi-way data by providing a set of time loadings for each assessor making it possible to study basic phenomena’s develop over time for the different assessors. Basically, PARAFAC can be compared to a bilinear PCA model, while PARAFAC2 follows the same multi-way approach with the advantage of overcoming shifts on time modes between the assessors.

Choosing the right method for data analysis is however difficult and depends on the type of data investigated ²²⁷. We believed the TI methodology combined with univariate and multivariate data analysis to be a safe and advanced way to capture differences between products, assessors and time profiles. TI parameters were therefore analyzed by classical univariate statistics and the results were then verified by multiway models such as PCA (paper II) or PARAFAC2 (paper I) using the full TI curves.

In part II ‘Spicy Meals, Appetite and Energy intake’ it was investigated how trigeminal stimuli and bioactive ingredients affect appetite and energy intake. Since the overall aim of the project ‘Meat as part of a meal’ was to promote culinary quality of catering meals the first meal study (paper III) was conducted in a canteen-like environment as this naturalistic setting was believed to give a more realistic measure of energy intake. Furthermore catering meals are most often ingested in a social context why subjects were placed at tables for six persons and were allowed to talk about everything but their opinions of the food. Social interactions and enhanced culinary context (e.g. restaurant-like environment) have been shown to induce greater energy intake than when the food is consumed individually in a simple environment ^{97,98,101,230}, an effect that might be caused by the prolonged meal duration due to the more favourable conditions ^{99,100}. Variations due to contextual factors were expected to be comparable on all test days and were therefore believed not to bias the energy intake. Five types of hot spices were added to five different meals that were designed to match the hot spices, to meet the expectation of a meal and to be balanced for flavours and basic tastes to make the meals as palatable as possible; however the final fixed meals varied in hotness, energy content and weight, which made it impossible to make comparisons between meals. One meal type instead of five would have made it possible to compare across spices and would have required the testing of only six interventions instead of ten, however using this approach could have compromised the palatability and the realism of the study. After ingestion of the fixed meal an ad libitum buffet-type dinner was served as ad libitum buffet resembles free-living conditions with minimal constraints on food selection, increases palatability, gives more reliable energy intake measures and has previously shown high reproducibility of ad libitum energy intake ^{70,231,232}. Disadvantages might however be that the macronutrient composition might vary between test days and thereby confound the

reproducibility of the ad libitum EI ^{70,232} as the satiating effects of proteins might be higher than for carbohydrates and fat ⁶⁰⁻⁶³. Before, during and after the meals subjects scored appetite, sensory specific desires and mood related sensations on 9-point category scales. These scales make the assessor's choice more limited than typical line scales such as 100 mm Visual Analogue Scales (VAS) however these scales were chosen since they are believed to be well suited for untrained consumers and be as sensitive to product differences as other scaling techniques ^{233,234}. Other limitations in the study design were that it was not possible to conclude if lack of effects of hot spices were caused by inadequate concentrations of active substances as only one concentration of each spice was used in the present study and it can not be excluded that the high protein content of the meals as well as the naturalistic settings might mask possible effects. Furthermore measuring energy intake and appetite only immediately before and after dinner might be too short time to observe the full effects of the hot spices.

Therefore the second meal study (paper IV) was conducted in a controlled lab environment. Hot spices were added to standardized breakfast and lunch meals as well as to ad libitum meals of fixed macronutrient composition. VAS was used for rating the subjective appetite sensations, sensory specific desires and liking as VAS scores are regarded as reliable measures with acceptable reproducibility and validity ^{70,235}. Furthermore VAS are the most commonly used method for measuring subjective appetite (hunger, fullness, prospective food consumption and satiety) as well as desire to eat (sweet, sour, bitter, salty, umami/savoury, fatty stimuli and hot) which makes it easier to interpret the results and compare them with outcomes from similar studies. The limitations of VAS are however that it can be difficult to standardize the method completely so all factors are kept constant for each time point on different test days, which is needed to make the measures reproducible ²³⁵. Furthermore biological day-to-day variations and methodological variation, cannot be distinguished ²³⁵. Since no objective measures can be directly compared with the subjective appetite sensations, the accuracy of VAS cannot be found and the validity can be difficult to assess ²³⁵. VAS scores are however often measured simultaneously with objective measures of energy expenditure, blood parameters (e.g. satiety hormones, glucose) and energy intake in research of appetite ²³⁵. In the present study it was planned to measure energy expenditure as well as the satiety hormones GLP-1, PYY and Ghrelin. These objective measures were never performed mainly due to lack of funding.

Paper I

Interactions between oral burn, meat flavor and texture in chili spiced pork patties evaluated by time-intensity



Due to restrictions from the publisher of the journal in which article I and II has been published, these articles are not available in this PDF. The articles can be found in:

Paper I:

Reinbach, H. C., Meinert, L., Ballabio, D., Aaslyng, M. D., Bredie, W. L. P., Olsen, K. et al. (2007). Interactions between oral burn, meat flavor and texture in chili spiced pork patties evaluated by time-intensity. *Food Quality and Preference*, 18, 909-919.
DOI: 10.1016/j.foodqual.2007.02.005

Paper II:

Reinbach, H. C., Toft, M., & Møller, P. (2009). Relationship between oral burn and temperature in chili spiced pork patties evaluated by time-intensity. *Food Quality and Preference*, 20, 42-49.
DOI: 10.1016/j.foodqual.2008.07.003

Paper II

**Relationship between Oral burn and Temperature in Chili Spiced Pork
Patties evaluated by Time-Intensity**

Due to restrictions from the publisher of the journal in which article I and II has been published, these articles are not available in this PDF. The articles can be found in:

Paper I:

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Manuscript I

Effects of capsaicin on taste cell signalling in animals

The following manuscript is based on preliminary data on 'Effects of capsaicin on taste cell signalling in animals' performed by Helene Christine Reinbach in collaboration with M.A. Lawson, D.A. Bruggemann and W.L.P. Bredie.

General introduction

Even though research of the trigeminal sense has focused on investigating how trigeminal stimulation from irritants interacts with gustatory sensations, no clear mechanism has yet been revealed. Most theories suggest that interactions between gustatory, olfactory and trigeminal stimuli occur via a central neural integration of sensory input in NTS, thalamic or cortex and/or via peripheral effects^{124,170-173,179-181}. Since some trigeminal afferents have projections to the NTS^{121,123,236,237}, capsaicin might be able to modulate responses of gustatory NTS neurons. Substance P emitted from trigeminal fibres have been found to modulate NTS responses to NaCl whereas capsaicin suppressed chorda tympani fibre responses to NaCl in animals^{238,239}. Furthermore gustatory NTS units responded to capsaicin although these responses were less evident than those elicited by the best taste, suggesting that either NTS units signal oral irritation or that capsaicin has gustatory properties¹⁸¹. The fact that capsaicin, piperine, zingerone and menthol can stimulate bitter taste when applied to the circumvallate region of the tongue in bitter tasters supports that irritants might possess taste qualities¹⁶¹⁻¹⁶³. However capsaicin significantly suppressed responses of gustatory NTS to the five basic tastes in both intact and trigeminal ganglionectomized rats, which indicates that the suppression is not mediated by a central trigeminal effect but influences taste processing at a more peripheral site¹⁸¹. Capsaicin was furthermore found to provoke plasma extravasations' and oedema in fungiform papillae, effects which might block the pore of the taste buds in taste papillae and thereby suppress taste perception^{181,240}. Alternatively capsaicin might cause changes in salivary flow or composition that change access of tastes to their receptors^{170,179}. However the latter theories do not explain why not all tastes are suppressed equally¹⁷². Therefore irritants themselves or substance P released from trigeminal fibres could act directly on taste cells to alter the gustatory responses^{161,181,240}. Capsaicin has been suggested to interact with G-protein- coupled transduction mechanisms for sweet, bitter and umami however no experimental evidence was found to support this theory²⁴¹. Therefore it was found of great interest to investigate if capsaicin can stimulate the bitter and sweet taste signalling in taste cells.

The aim of this study was therefore to investigate how stimulation with capsaicin affects bitter and sweet taste signalling in taste cells. Rat taste cells were used as a model system and the ratio between ERK2 and phospho-ERK2 protein was used as a marker of taste signalling (experiment 5.1). A second experiment was performed with pig tongue tissue, which was stimulated with varying capsaicin concentrations to investigate how capsaicin affects the epithelia and filiform papillae (experiment 5.2).

Experiment 5.1

Methods

Stimuli solutions

Capsaicin (≥ 99 % HPLC, Fluka), Pluronic F-68 (10 % solution, GIBCO, Invitrogen), Linolenic acid (99 %, Sigma)

Eight solutions were prepared in eppendorf tubes:

1. Solvent (**F**) (water with 0.2 w/w % pluronic F-68)
2. Solvent + capsaicin (**FC**) (100 μ M capsaicin, 0.2 w/w % pluronic F-68)
3. Quinine (**Q**) (0.001 M quinine-HCl, 0.2 w/w % pluronic F-68)
4. Quinine + capsaicin (**QC**) (1 mM quinine-HCl, 0.2 w/w % pluronic F-68, 100 μ M capsaicin)
5. Sucrose (**S**) (0.3 M sucrose, 0.2 w/w % pluronic F-68),
6. Sucrose + capsaicin (**SC**) (0.3 M sucrose, 0.2 w/w % pluronic F-68, 100 μ M capsaicin)
7. Linolenic acid (**L**) (0.5 w/w % linolenic acid, 0.2 w/w % pluronic F-68)
8. Linolenic acid + capsaicin (**LC**) (0.5 w/w % linolenic acid, 0.2 w/w % pluronic F-68, 100 μ M capsaicin).

Tongue tissue preparation

Four Sprague-Dawley rats with an age of three month were killed and the tongues were removed and tissue was divided into segments with a scalpel as illustrated in figure 5.1. The sides of the tongue were used for stimulation with sweet and bitter taste (sucrose and quinine) as well as taste and capsaicin. Back and tip of the

tongues were used for optimizing the methods of the cellular assay developed by Lawson et al.²⁴².

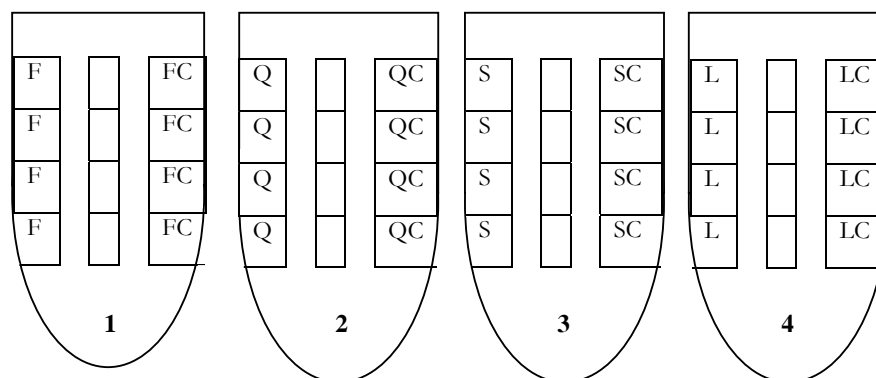


Figure 5.1 Sectioning of Sprague-Dawley rat tongues. Tongue 1 was the control tongue stimulated with solvent (F) and solvent + capsaicin (FC), tongue 2 with quinine (Q) and quinine + capsaicin (QC), tongue 3 with sucrose (S) and sucrose + capsaicin whereas tongue 4 was stimulated with linoleic acid (L) and linoleic acid + capsaicin (LC).

The tongue tissue was then exposed to solutions with either control solvent (F), tastes (Q, S) and fatty acid (L) or tastes, fatty acids and solvent combined with capsaicin (FC, QC, SC and LC) for 10 secs. The tissue samples were then fixed in 4 % paraformaldehyde for 1 hr, frozen in Tissue-Tec (Sakura Finetek, NL) by liquid nitrogen and stored at -80 °C. The frozen tissue samples were cut into 10 µm slices on a microtome (2800 Frigocut N, Reichert-Jung) and placed on object glasses (PolysineTM, Menzel-Glaser, 25x75x1 mm) surrounded by a hydrofob ring (Dakocytomation).

Immunostaining

Primary antibodies: Mouse anti-MAP Kinase (ERK1+ERK2) and Rabbit anti-phospho-MAP Kinase (P-ERK1+ERK2) (Zymed, Invitrogen).

Secondary antibodies: Labeled Donkey Anti-Mouse IgG and Labeled Donkey Anti-Rabbit IgG Antibodies (Molecular Probes, Invitrogen).

The tongue tissue slices were rehydrated in PBS, fixed in 4 % paraformaldehyde for 15 mins and washed in PBS three times. Tissue was incubated with block proteins (8 % BSA in PBS buffer) for 40 mins. Tissue was then incubated with primary antibodies (dilution 1:100 in 2 % BSA in PBS) for 1 hr at room temperature, washed three times in PBS and incubated with secondary antibodies (dilution 1:1000 2 % BSA in PBS) and nucleus staining dye DAPI (1:2000 2 % BSA in PBS) for 1 hr at room temperature. After washing three times in PBS, tissue was mounted in a droplet of fluorescent mounting medium, covered with a cover glass and frozen at -20 °C until microscopy inspection. A negative control was performed using the same procedure described above however leaving out incubation with primary antibodies.

Fluorescence microscopy

Immunostaining of ERK1+2 and phospho-ERK1+2 in conjunction with rhodamine were used to visualize cell membranes whereas DAPI was used to identify cell nuclei. A Leica DM IR B fluorescence microscopy and the Image Pro Plus software were used for visualizing signal transduction in taste buds. The tissue samples with the best cross-sectional cuts through circumvallate and filiform papillae were chosen for microscopy. The best images were transferred to Photoshop to adjust the brightness and the contrast.

Results

The rat tongue tissue slices were analysed under the fluorescent microscopy. The ratio between ERK1+2 and phosphorylated ERK1-2 was used as a measure for signalling in taste buds and filiforms. The thin cutting of the tissue seemed to spoil and fold the tissue, which made it difficult to study the structures in the tissue stimulated with quinine and linolenic acid (Q, QC, L, LQ). The control samples (F, FC) revealed that filiform papillae had a bended shape in the presence of capsaicin, unlike the clear peaks that are characteristic for these papillae. However the staining clearly illustrated that capsaicin activated ERK1-2 signalling in both filiform papillae and supporting cells surrounding the taste buds (figure 5.2). Furthermore taste buds were activated by sucrose and this activation was lower than when the taste buds were stimulated with both sucrose and capsaicin (figure 5.2).

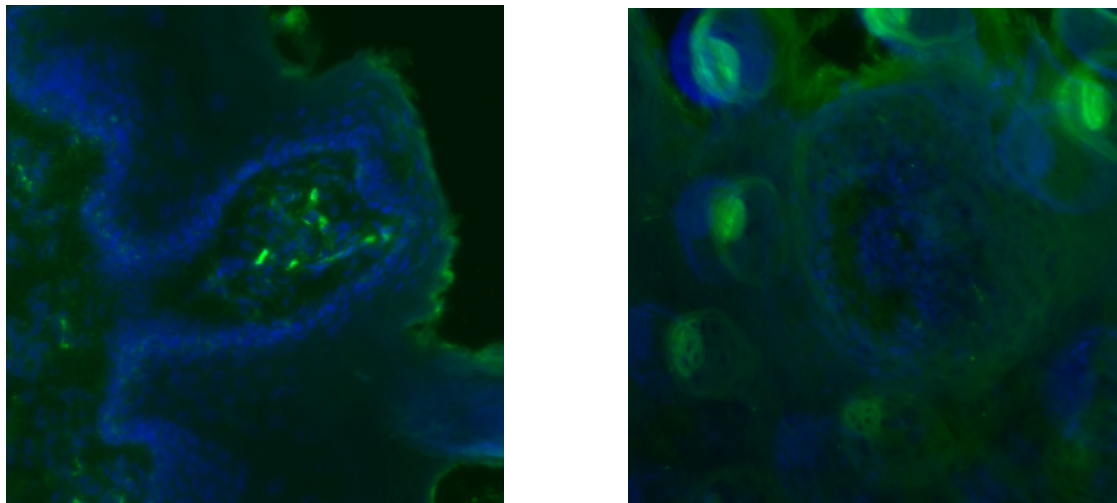


Figure 5.2 Confocal laser microscopy of taste buds stimulated with a) Sucrose and b) Sucrose and capsaicin. Green staining represents phospho-ERK1+2 proteins whereas blue colour represents nucleus (DAPI) staining.

Experiment 5.2

Methods

Stimuli solutions

Pluronic F-68 (10 % solution, GIBCO, Invitrogen), (Capsaicin ≥ 99 % HPLC, Fluka).

Eight solutions (Capsaicin diluted in water with 0.2 w/w % pluronic F-68) were prepared with the following capsaicin concentration:

0 μ M Capsaicin

3.125 μ M Capsaicin (low)

6.25 μ M Capsaicin

12.5 μ M Capsaicin

25 μ M Capsaicin (medium)

50 μ M Capsaicin

80 μ M Capsaicin

100 (high) μ M Capsaicin

Tongue tissue preparation

A pig tongue was taken out during daily slaughtering at the slaughter house of Roskilde and the tongue tissue was divided into segments with a scalpel as illustrated in figure 5.1. Pieces from the sides of the tongue were stimulated with eight different capsaicin concentrations for 10 secs. The tissue samples were then fixed in 4 % paraformaldehyde for 1 hr, frozen in Tissue-Tec (Sakura Finetek, NL) by liquid nitrogen and stored at -80 °C.

*Filiform test***Whole tissue**

Primary antibodies: Mouse anti-MAP Kinase (ERK1+ERK2) and Rabbit anti-phospho-MAP Kinase (P-ERK1+ERK2) (Zymed, Invitrogen).

Secondary antibodies: Labelled Donkey Anti-Mouse IgG and Labelled Donkey Anti-Rabbit IgG Antibodies (Molecular Probes, Invitrogen).

Three pieces of pig tongue tissue (I: low capsaicin, II: high capsaicin and III: high capsaicin) were defrosted and washed in water and some of the muscle tissue was cut off. The tissues were stained as described in table 5.1.

Table 5.1 Immunostaining performed on three pig tongue tissue samples (I, II, III).

	I (low capsaicin)	II (high capsaicin)	III (high capsaicin)
Saponin	50 µg/ml	50 µg/ml	5 µg/ml
Primary staining	1:100 Mouse anti-MAP Kinase (ERK1+ERK2) 1:1000 Rabbit anti-phospho-MAP Kinase (P-ERK1+ERK2)	1:100 Mouse anti-MAP Kinase (ERK1+ERK2)	1:100 Mouse anti-MAP Kinase (ERK1+ERK2) 1:1000 Rabbit anti-phospho-MAP Kinase (P-ERK1+ERK2)
Secondary staining	1:1000 Labelled Donkey Anti-Mouse IgG 1:1000 Labelled Donkey Anti-Rabbit IgG Antibodies 1:100 WGA	1:1000 Labelled Donkey Anti-Mouse IgG 1:100 WGA	1:1000 Labelled Donkey Anti-Mouse IgG 1:1000 Labelled Donkey Anti-Rabbit IgG Antibodies

Tissues were incubated in PBS with 5 or 50 µg/ml Saponin for 2 hrs at room temperature and washed three times in PBS. Tissues were blocked in 4 % BSA in PBS buffer for 1 hr at room temperature. Tissues were then incubated with primary antibodies (dilution 1:100 and 1:1000 in 2 % BSA in PBS) for two hrs at room temperature. After washing five times in PBS, the tissues were incubated with

secondary antibodies (dilution 1:100 and 1:1000 in 2 % BSA in PBS) and wheat germ agglutinin (WGA) (Alaxa fluor^R conjugate) (1:100 2 % BSA in PBS) over night.

Pig tongue slices

A frozen tissue sample stimulated with 50 μ M capsaicin was cut into 40 μ m slices on a microtome and placed on object glasses (PolysineTM, Menzel-Glaser, 25x75x1 mm) surrounded by a hydrofob ring (Dakocytomation pen). Immunostaining was performed as described under experiment 5.1.

Results

The whole pig tongue tissue samples (table 5.1: I, II and III) were analysed under the confocal laser fluorescent microscope. Sample III contained spikes with clear phosphor-ERK1-2 signalling, which could resample activity in filiforms due to high capsaicin concentration. Several structures were found on the three samples however no structures characteristic for taste buds. We therefore changed strategy and focused on slices of pig tongue tissue, which might be easier to handle under the confocal laser microscope. However the staining revealed that the whole ephithelium was full of ERK1+2 activities even in the nucleus of the cells.

General discussion

In the present study a cellular assay was performed with rat tongue tissue to study taste signalling in the presence of taste (quinine or sucrose) and in taste/capsaicin mixtures. The ratio between ERK1-2 protein and its phosphorylated form was used as a measure for taste signalling. Our results indicate that capsaicin could suppress sweet taste signalling. In accordance, Costa et al found that suppression of sweet taste by capsaicin was mediated through a TRPV1-independent mechanism affecting activity in animal taste cell ²⁴³. In humans TRPV1 has not yet been identified in association with gustatory neurons or taste buds of the chorda tympani nerve ¹⁶¹ suggesting that taste suppression by capsaicin could also occur via a TRPV1-independent mechanism in humans. Unfortunately either the cutting method used to slice the rat tissue or the presence of capsaicin seemed to spoil the epithelium especially the filiforms papillae in the present study, since filiforms with an uncharacteristic bended shape were observed in the presence of capsaicin. The

cellular assay was also performed on pig tongue that was either cut in pieces (several mm) or in 40 μ M thick slices. However it was difficult to identify taste buds and filiforms in pig tongue by immunostaining of ERK1+2 proteins. A complex anatomy of the pig tongue as described by Kumar et al.²⁴⁴ combined with high activity of ERK1+2 proteins in several cell structures suggested that the cellular assay used was not appropriate to determine taste signalling in pig tongue. Therefore we suggest that the cellular assay should be optimised. The staining could be more targeted to signalling of taste and trigeminal stimuli by including staining of taste and irritant receptors as well as important signalling and nerve mediators such as cAMP, Phospho-lipase C and gustducin. Since capsaicin has previously been found to activate bitter taste it would be highly interesting to investigate if capsaicin can activate a subset of bitter taste receptors or if capsaicin-sensitive taste neurons can be identified by immunostaining¹⁶¹. Furthermore optimisation test could be performed with different capsaicin concentrations as well as different sample preparations of the tissue to reveal how these factors influence tissue damage. The limitation of the method is that we only get a snapshot of what is going on in the cell and a possible delay in the responses to taste and irritants might not be caught. However since both signal transduction pathways in taste cells and possible interactions with the trigeminal sense are still poorly understood, we believe that the present cellular assay in a more developed and optimised form could reveal if interactions between gustation and the trigeminal sense occur on taste cell level.

Paper III

**Effects of hot spices on energy intake, appetite and sensory specific desires
in humans**

Effects of Hot Spices on Energy Intake, Appetite and Sensory Specific Desires in Humans

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Abstract

The present study investigated how hot spices affect energy intake, appetite and desires. 40 participants received five different meals of fixed portion sizes, which were served with or without hot spices (\pm chilli pepper, \pm horseradish, \pm ginger, \pm mustard and \pm wasabi), and then followed by a buffet. Appetite, liking, mood and desire to eat sweet, sour, fatty, salty, bitter and hot foods were scored on 9-point category scales and total food intake (kJ and g) was measured. Chilli pepper, horseradish, ginger, mustard and wasabi did not affect energy intake (kJ); lack of effects could be caused by inadequate concentrations of hot spices and limitations in the study design. Desire to eat sweet foods was increased by chilli whereas desire to eat salty products was decreased by mustard. These findings suggest that hot spices can induce changes in sensory specific desires. More controlled studies could however reveal if desires are reflected in food choice and eating behaviour, as well as determine optimal doses of hot spices to gain beneficial effects on appetite and energy intake.

Keywords: Energy intake, Appetite, Desires, Chilli, Horseradish, Ginger, Mustard, Wasabi, Meal, Humans.

Introduction

Hot spices like chilli peppers, horseradish, ginger, mustard and wasabi are commonly used in many cuisines to stimulate our senses with exotic flavours and burning, hot sensations, which can give meals an exciting twist. Apart from enhancing the pleasure of eating, some of these spices also provide nutritional value and have anti-oxidant as well as antibacterial properties [1,2]. Capsaicin, the predominant pungent substance in chilli peppers, has shown promising effects on energy expenditure and appetite in humans [3-8]. Human studies have revealed that high doses of chilli peppers suppress appetite and reduce energy intake by inducing a change in macronutrient intake [5,8] and largest reductions in energy intake are observed when the chilli pepper is administered orally compared to gastrointestinal stimulation [5,9]. Increased activity of the sympathetic nervous system (SNS) after ingestion of red pepper suggests that the reduction in energy intake could be due to the anorectic effect of catecholamine's [8,10]. Overall these findings illustrate promising short term effects of chilli peppers on energy intake and appetite. Due to these beneficial health effects, herbal supplements with hot spices such as ginger and capsaicin have been marketed and sold as health food supplements for decades. Since obesity is now a global problem leading to

increased risk of developing lifestyle diseases (e.g. Type II diabetes, hypertension and cardiovascular heart diseases), it is of great interest to explore if hot spices, could be an attractive supplements to common weight-loss strategies using increased physical activity and strict diet control to facilitate weight loss.

Based on this evidence it was hypothesized that hot spices, might suppress energy intake and appetite. Five hot spices (chilli peppers, horseradish, ginger, mustard and wasabi) were added in tolerable amounts to different well-composed fixed meals, balanced in sour, sweet, fat, bitter and salty properties to make the meals palatable and to avoid fatigue due to repeated exposure of the meals. All five fixed meals were served as a starter with or without hot spices followed by an ad libitum buffet. The effect of hot spices on appetite, liking, mood, sensory specific desires and ad libitum energy intake were measured with 9-point category scales.

Methods

Participants

40 subjects (17 men and 23 women) with a mean age of 24.6 ± 2.5 years (mean ± SD), mean BMI of 22.5 ± 2.7 kg/m² were recruited from Faculty of Life Sciences, University of Copenhagen. Subject characteristics are summarized in Table 1. Criteria for the subjects included to be in good health, be between 19 and 30 years, not suffer from any food allergies and like to eat hot spices and pork meat. To confirm that the inclusion criteria were met, a questionnaire on health status, meal pattern and food intake was developed inspired by the questionnaire on food intake and lifestyle created by the Danish Cancer Society [11]. Subjects were classified into eaters of chilli (ingestion of chilli once a week or more) and non-eaters of chilli (less than once a week) based on the questionnaire developed by Lawless et al. [12], which was used in the present study with a few modifications as described in Reinbach et al. [13]. The subjects were asked to fill out the questionnaires on the first experimental day and their heights and weights were measured to calculate body mass index (BMI).

Subjects were allowed to stick to their own breakfast and lunch as long as they ate the same portions and types of food on every test day. We chose not to standardize the breakfast and lunch for all subjects since we wanted the experiment to be as close to daily routines as possible. Eating and drinking, with the exception of water, were not allowed three hours prior to a meal session and smoking and coffee were prohibited for one hour before. Furthermore subjects were asked to abstain from intense physical activity the day before an experiment.

Procedure

Each subject participated in a crossover meal study where ten test meals, corresponding to 5 different meals ± 5 different hot spices (chilli pepper, horseradish, ginger, mustard and wasabi) were consumed twice a week, separated by at least 48 hours, for five consecutive weeks. It was aimed for to create a realistic eating environment similar to what catering food services might offer in schools, hospitals or company canteens. Therefore the meal study took place in surroundings, mimicking a canteen dining room with coloured napkins and flowers used to create a good atmosphere. Participants could freely choose a seat from four different tables and were allowed to talk about everything but their opinions of the food. At each meal session all participants received the same meal of fixed portion size at 6.00 p.m.; half of the participants were randomized to a spicy meal and the other half to a non-spicy meal. All subjects were asked to finish the meal completely with no leftovers allowed. 30 min after the fixed meal was served, subjects could freely choose from an ad libitum buffet containing all the food components from the fixed meal. The same food components were served for the ad libitum buffet as for the fixed meal to mimic serving of the 'warm dish of the day' in a canteen. Each subject noted down his or her food choices from the buffet in

a personal schedule before eating ad libitum until satiation. Total food (g) and energy intake (kJ) of both the fixed meal and the ad libitum buffet as well as total water intake (g) were measured by weighing the food components before and after serving and then calculate the difference. Participants were asked to spend one hour per meal session and received a bottle of red wine as payment.

Ratings of Appetite, mood, liking and sensory specific desires

All ratings were scored on 9-point category scales since category scales are believed to be well suited for untrained consumers and are as sensitive to product differences as other scaling techniques [14,15]. Participants were asked to score their appetite as *hunger* ranging from 'not at all hungry' at 1 to 'very hungry' at 9, *wanting to eat more* from 'very weak' to 'very strong', *prospective food consumption* from 'nothing at all' to 'a very large portion', *fullness* from 'not at all full' to 'very full' and finally *satiety* from 'not at all satiated' to 'very satiated'. Appetitive ratings were made before the fixed meal, every three minutes during the fixed meal and again after the buffet (meal termination). Liking defined as *immediate pleasure from eating*, was anchored with 'not at all' and 'very much' and scored every third minute during the fixed meal and at meal termination. Mood conditions *well-being* ('very uncomfortable' to 'very comfortable'), *mood* ('very bad' to 'very good'), *levels of relaxation, stress and exhaustion* ('not at all' to 'very ') were scored before and after the fixed meal and again after the buffet. To investigate how hot spices affect the sensory specific desires, participants scored their desires to eat sour, sweet, fat, bitter, salty and hot stimuli after the fixed meal and again after the buffet ranging from 'not at all' to 'yes very much'. Desire was defined as the intrinsic motivation to engage in eating a food, now or in the near future [16]. The sensory properties of the meals were evaluated with respect to sourness, sweetness, fatness, bitterness, saltiness and hotness, all scored from 'not at all' and 'very much' after the fixed meal.

Table 1. Subject characteristics (mean± Standard deviation)

	Men	Women	All
<i>N</i>	17	23	40
<i>Age (years)</i>	25.4±2.6	24±2.2	24.6±2.5
<i>Body weight (kg)</i>	76.1±8.4	63.0±12.1	68.5±12.4
<i>BMI (kg/m²)</i>	23.3±1.8	21.8±3.0	22.5±7
<i>Eater of chili</i>	7	8	15
<i>Non-eater of chili</i>	10	15	25

Meals

All five different fixed meals were developed consisting of reasonable amounts of pork meat (50-100g), vegetables (50-100g), a salad (50-150 g) and a dressing (30-50g) to meet the expectation of a meal. Generally the evening dinner contributes to 25-35 % of total daily intake [17]. Based on this assumption the fixed meals were composed to provide less than 2350 kJ corresponding to approx. 25 % of daily energy need for an average sedentary women of 18-30 years to ensure that all subjects would eat from the ad libitum buffet after the fixed portioned meal. Subjects were asked to eat all the food served during the fixed meal. The amounts and energy contents of the fixed meals and the portion sizes of the buffet are given in Appendix 1. The energy content of the meals was calculated from the nutrition information on the food items or from a standard Danish nutritional table [18]. The food components in each meal were chosen so that sour (U), sweet (W), bitter (B), salty (S), fat (F), crunchy (C) and \pm hot (H) stimuli were presented in all the meals. The amount of hot spices used was determined from the recommended serving size of the product and from several tastings of the spiced dressings, which were performed to find the highest doses, perceived as hot but still possible to ingest (0.6 g chilli pepper, 10 g horseradish, 20 g ginger, 19.2 g mustard) (hotness ratings of ≥ 7 on a 9-point scale). Chilli pepper, ginger and mustard were added to the dressing of the meal and placed on the meat, the grated horseradish to the salad and the wasabi to the rice in each respective meal.

Data analysis/Statistical analysis

The results are presented as the mean \pm standard error (SE). Repeated analysis of the appetite scores was performed by linear mixed statistical models (proc mixed) in SAS (SAS Institute, Inc., Cary, NC, USA) using a model with session and subjects as random effects, treatment, gender, time and eater vs. non-eater as fixed factors whereas BMI, age, baseline appetite and liking were used in the model as covariates. Proc mixed analysis of total energy intake (kJ) and total food intake (g) ingested during the fixed meal and the buffet were evaluated for significant effects of gender, eater vs. non-eater of chilli and treatment (fixed factors) as well as BMI, age and baseline energy intake (kJ and g) (covariates) using session and subjects as random effects. Similar mixed models were made for desires to eat scores (fixed factors: gender, eater vs. non-eater of chilli, serving (after fixed meal and after buffet), desire and treatment; covariates: BMI and age; random effect: subjects) and mood (fixed factors: gender, eater vs. non-eater of chilli, serving and treatment; covariates: BMI, age and baseline mood; random effects: session and subjects). By using subjects as random effect the mixed model can handle judge-to-judge variation in a proper way so the variability due to treatment and conditions can be studied and general conclusions can be made from the test sample (n=40 participants). The mixed model can handle unbalanced factors such as gender, imbalance due to missing values and do model correlations in complex models with covariates.

Significance was set at a p-value of <0.05 . Post hoc pairwise comparison across treatments was performed by using Tukey-Kramer's test (adjusted p adj p). Comparisons were only made between the spicy and non-spicy version of the same meal so that meal 1 was compared to meal 1 with chilli, meal 2 with meal 2 with horseradish etc. Overall trends in scores of appetite, mood, energy intake, desires and sensory properties of the food were studied using principal component analysis (PCA) with Latentix software (LatentiX™ 2006, Latent5, Denmark).

Results

Energy intake (kJ and g)

Adding spices to the meals had only minor effects on total energy intake (kJ) (Figure 1a) and no effect on total food intake (g) (Figure 1b). Adding spices to the meals had no effect on water intake (data not shown).

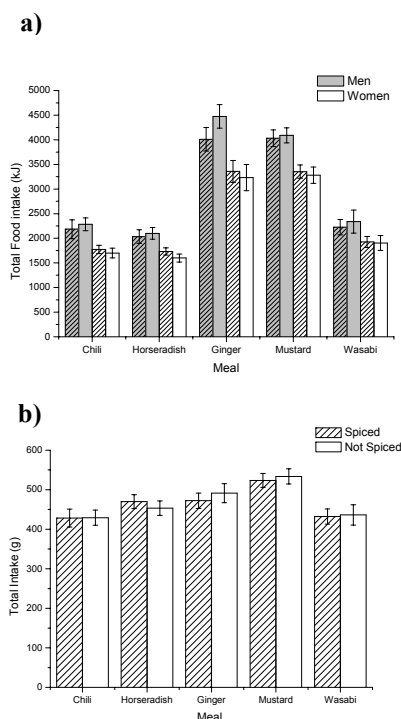


Figure 1 Mean \pm SE of total food intake in **a)** in kJ for men (gray bars) and women (white bars) and **b)** in g. Bars with pattern indicate the spiced meals and bars without pattern indicate non-spiced meals.

Appetite

Analysis of the repeated measures of appetite revealed that adding spices to the meals had no significant effect on hunger or satiety. However, adding wasabi to meal 5 significantly increased prospective food consumption ($p=0.03$) (data not shown). Adding chilli to meal 1 significantly increased

wanting to eat more in women (gender*treatment: $p=0.003$, pairwise comparison meal 1 \pm chilli: $p=0.01$; adj $p=0.50$) whereas ginger decreased wanting to eat more in men (pairwise comparison meal 3 \pm ginger $p=0.009$; adj $p=0.50$) (data not shown).

Mood

Adding spices to the meals had no effect on mood, relaxation and satisfaction although adding ginger (pairwise comparison meal 3 \pm ginger: $p=0.005$) and wasabi (pairwise comparison meal 5 \pm wasabi: $p=0.05$) seemed to reduce well-being (data not shown).

Sensory specific desires

Chilli increased the desire to eat sweet in meal 1 ($p=0.041$; adj $p=0.99$), mustard decreased the desire for salty stimuli in meal 4 ($p<0.003$; adj $p=0.71$) and all spices except ginger reduced the desire for hot stimuli (treatment*desire $p<0.0001$, pairwise comparison \pm spices $p<0.02$; $p<0.99$) (Figure 2). Generally adding spices to the meals (chilli to meal 1, mustard to meal 4 and wasabi to meal 5 ($p<0.0001$; adj $p=0.01$) significantly reduced the desire to eat after the fixed meal ($p<0.01$; adj $p<0.51$) whereas no effect of adding spices to the meals was found after the buffet.

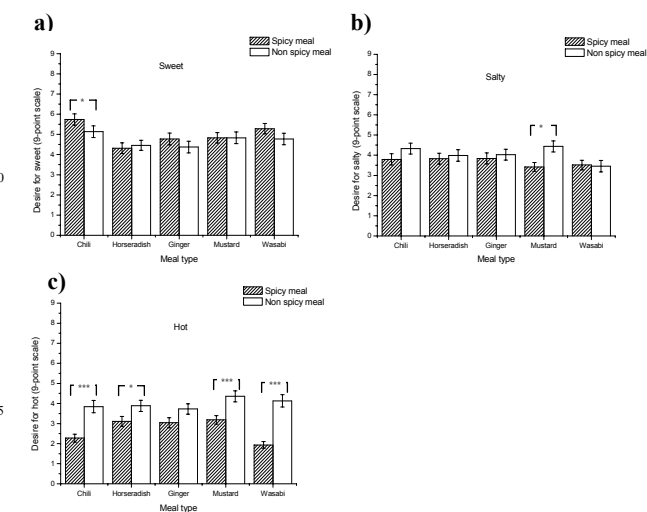


Figure 2 Means \pm SE of desire to eat scores for sweet, salty and hot stimuli comparing the spicy meal (dashed bars) with the non-spicy version of the meal (white bars). Notation of significance: * (5 %), ** (1 %), *** (0.1 %)

Liking and hotness

Average liking scores revealed that all meals were generally liked (liking > 5) by the subjects, with exception of meal 5 with wasabi (average liking score 4.9). Adding mustard significantly increased liking of meal 4 (from 6.5 to 7.2) during ingestion of the fixed meal (treatment*time: $p<0.0001$) whereas none of the other spices affected liking. All the

spiced meals were perceived significantly hotter than the non-spiced meals (data not shown) but the different spiced meals were not perceived to be equally hot (average oral burn for horseradish and ginger: 5.5, mustard: 6.1, chilli: 7.8 and wasabi: 8.4) and the meals spiced with horseradish, ginger and mustard were perceived to have less burn than aimed for. Generally, liking did not change during progression of the fixed meals, although a decrease in liking was observed with meal 5 over time ($p<0.031$). For most meals (meal 1 +chilli, meal 2 \pm horseradish, meal 3 \pm ginger, meal 4 + mustard, $p<0.04$) liking tended to increase after the buffet (50 min) when compared to liking during the fixed meals.

Eater versus non-eaters of chilli

Overall the classification of eaters and non-eaters was found to be of less importance when studying the effect of hot spices on energy intake, appetite, mood and sensory specific desires. It was observed that eaters of chilli tended to eat less (kJ and g) than non-eaters when the food was not liked (liking scores < 5) whereas eaters of chilli had a higher food intake than non-eaters when the food was liked (liking scores > 5) (liking*Eater_non-eater: kJ $p=0.02$; g $p=0.04$, data not shown).

Relationship between appetite, mood, energy intake, sensory specific desires and sensory properties of the meal

A score plot of PC1 and PC3 showed that PC1 separated samples according to meal types (Figure 3) but with low explained variance (18.732 %). Overall tendencies found from studying scores, loadings and bi-plot showed that the meal 1 \pm chilli and meal 2 \pm horseradish were perceived as sour, sweet and bitter and that these meals induced desire to eat more sweet, fatty, bitter and salty foods. The meal 3 \pm ginger, meal 4 \pm mustard and meal 5 \pm wasabi were perceived more fatty and salty and induced desire to eat sour food. These data indicate that the more energy dense meals (meal 3 \pm ginger and meal 4 \pm mustard) lead to greater satisfaction and fullness than the less energy dense meals (meal 1 \pm chilli and meal 2 \pm horseradish). Interestingly the low energy dense meal 5 \pm wasabi led to the same satisfaction as the more energy dense meals. Furthermore, tendencies towards better mood, well-being and more relaxed atmosphere were observed after the low energy dense foods (meal 1 \pm chilli, meal 2 \pm horseradish) whereas meal 3 \pm wasabi, meal 4 \pm ginger and mustard led to higher stress and more exhausting sensations.

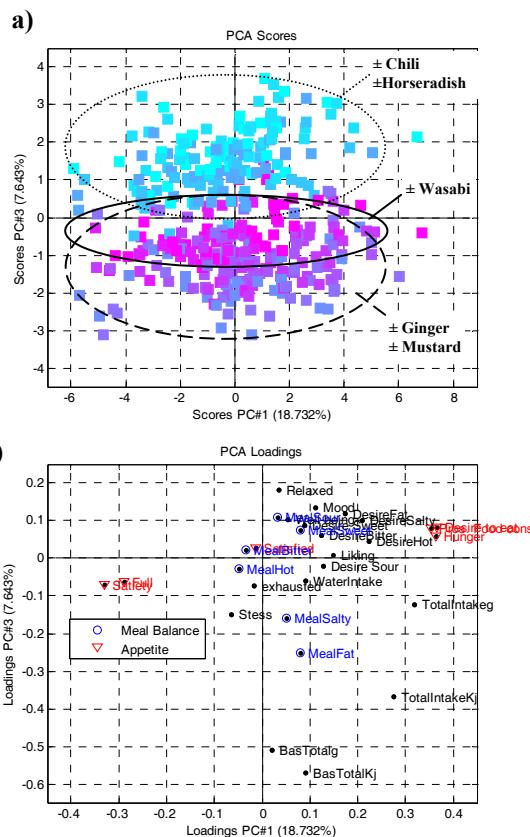


Figure 3 PCA plot of a) scores and b) loadings performed on all samples for appetite (red), mood, food intake (kJ and g), sensory specific desires and sensory properties of the meals (blue). Data was autoscaled and full cross validated. Ellipses represent three clusters in the data. Cluster 1: meals \pm chili, \pm horseradish, cluster 2: \pm wasabi and cluster 3: \pm ginger and \pm mustard.

Discussion

As chilli pepper previously has been found to reduce energy intake and appetite [5,8,9], we hypothesized that other hot spices might exert similar effects.

The present study revealed that adding chilli, horseradish, ginger, mustard or wasabi to a fixed meal had no evident effect on energy intake at a succeeding free choice buffet and only minor effects of adding hot spices were observed on appetite scores. Adding wasabi to a meal increased prospective food consumption and reduced liking during progression of the meal, suggesting that wasabi might induce a wanting for something other than the wasabi meals. Even though liking increased by adding mustard to the fixed meal, no increase in total energy or food intake was observed. In accordance palatability of the foods has previously been found to explain only minor variations in food intake and food choices when tested in naturalistic settings [19-21].

The hot spices were added in different amounts aiming for tolerable hot meals, but the the varying hotness for the different meals made it difficult to relate the meals to each

other. Since only one concentration was tested for each spice it is not clear whether the lack of effect on appetite and energy intake is due to inefficient doses of hot spices used or if the bioactive ingredients in horseradish, mustard and wasabi (allylisothiocyanates (AITCs)) as well as ginger (gingerols, zingerone and shogaols) do not affect appetite and energy intake. Furthermore it is possible that the effects of hot spices on energy intake and appetite-related sensations are partly masked by the high protein content of the meals as proteins are known for their high satiating effect [22-25]. Such masking effect could make it complicated to evaluate whether or not the amount of spices used was too low.

The hot spices used in this study had only minor effects on the mood-related sensations as ginger and wasabi reduced well-being. The fact that the least liked meal (meal spiced with wasabi) reduced well-being whereas the most liked meal (meal spiced with mustard) reduced stress suggests that liking of the meal is a strong determinant for mood related sensations. Likewise highly pleasant-tasting foods has been shown to exert small but positive changes in mood [26]. Not being accustomed to the oral burn from ginger and wasabi might also have affected well-being as liking for spicy foods is believed to be acquired after several exposures [27-29]. We however hypothesized that mood-related sensations could be affected by adding hot spices to the meals since ingestion of chilli, have been proposed to cause release of endorphins, the natural morphine-like pain reliever, which could result in a kick or rush sensation [27]. The lack of an effect of hot spices on mood related sensations could be due to the relatively low dosage used.

Chilli, horseradish, mustard and wasabi reduced the desire to eat hot stimuli, suggesting that sensory specific satiety towards hot stimuli is induced by these spices. Furthermore addition of mustard to a meal decreased the desire for salty stimuli and as the PCA analysis revealed the meals with and without mustard were characterized by being salt and fat, the decrease in desire to eat salty stimuli after a mustard spiced meal could be a sign of sensory specific satiety. Addition of chilli was on the other hand found to increase the desire to eat sweet foods even though the meal with and without chilli was characterized by being sour, sweet and bitter. This finding could indicate that hot spices might affect food desires. Previously chilli peppers have been found to increase the perceived oiliness of meals high in carbohydrate to levels comparable to high fat meals [7], which further support that hot spices affect food desires and sensory properties of a meal. Furthermore, adding spices to the fixed meals had no effects on the desire to eat ratings after the buffet suggesting that subjects reach a plateau of satisfaction and satiation with a free choice buffet, where desires for the basic food stimuli are lower and independent of the spices given at the fixed meal. Sensory specific desires might therefore have greatest importance when subjects are hungry insuring that a variety of foods with different sensory properties and nutritional value are consumed. Overall liking tended to be enhanced during the buffet when compared to the fixed meals, suggesting that

sensory specific satiety does not set in during a free choice buffet. For future experiments it would be interesting to investigate further how hot spices affect food desires and eating behaviour, as such knowledge could be useful in developing healthier and more satisfying catering meals for consumers.

The present study was conducted in a naturalistic setting mimicking an eating situation in a catering canteen. Social interactions and enhanced culinary context (e.g. restaurant-like environment) have been shown to induce greater energy intake than when the food is consumed individually in a simple environment [30-33], an effect that might be caused by the prolonged meal duration due to the more favourable conditions [34,35]. Even though these contextual factors might have affected energy intake it was believed that variations due to contextual factors would be comparable on all test days and that naturalistic settings would give a more realistic measure of energy intake as people often eat together in social settings. However it cannot be excluded that the effects of hot spices on energy intake and appetite might have been too small to overcome variations due to contextual factors as well as day-to day variations in hunger sensations and physical activity, etc. Furthermore, the five different hot spices investigated were served in five different meals designed to match the hot spices, to meet the expectation of a meal and to be balanced for flavours and basic tastes to make the meals as palatable as possible. To meet these expectations the final fixed meals varied in hotness, energy content and weight, which made it difficult to make comparisons between meals. To measure energy intake it was chosen to serve a buffet-type meal for dinner as this method previously has shown high reproducibility of ad libitum energy intake [36]. The advantage of buffet-type meals is that they resemble free-living conditions with minimal constraints on food selection, they increase palatability and give more reliable ad libitum energy intake measures however the macronutrient composition might vary between test days and thereby confound the reproducibility of the ad libitum energy intake [37,38] as the satiating effects of proteins might be higher than for carbohydrates and fat [22-25]. In the present study the buffet was arranged with small serving sizes of the same meal components as were served in the fixed meal, an approach that differs from the traditional controlled experiments where subjects are offered a variety of new foods ad libitum. Our approach might have made it more difficult to interpret the results since subjects unconsciously could have standardized their food intake by counting the number of small servings ingested during the buffet. Additionally, observing energy intake and appetite only immediately before and after dinner might be too short time to observe the full effects of hot spices. Therefore, more controlled studies with standardized meals served in lab environments might be needed to fully elucidate if bioactive ingredients (e.g. capsaicin, AITC, gingerols and shogaols) can induce suppressive effects on energy intake and appetite and by which mechanisms, sensory, cognitive as well as physiological, these effects can be explained. It would be

crucial to find the adequate doses of these bioactive ingredients to gain beneficial effects on energy intake and appetite to evaluate if these effects appear in quantities that might aid weight loss or weight maintenance.

In summary, ingestion of chilli pepper, horseradish, ginger, mustard and wasabi in tolerable doses with a meal had no evident effect on energy intake, appetite, mood and sensory specific desires at a succeeding free choice buffet. The minor effects of hot spices that were observed on appetite, mood and sensory specific desires could most likely be explained by changes in liking, the concentration of spices used rather than the spice itself as well as development of sensory specific satiety. It is not clear if the lack of effects of hot spices is caused by inadequate concentrations of active substances used in the present study or if the high protein content of the meals and the naturalistic settings have masked possible effects.

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Appendix 1. Composition and serving size (g) of the fixed meals and the buffet as well as energy content of the fixed meal (kj and energy percentages). Sour (U), sweet (W), bitter (B), salty (S), fat (F) and crunchy (C) stimulus were presented in all the meals

Food	Meal 1 ± Chilli pepper		Meal 2 ± Horseradish		Meal 3 ± Ginger		Meal 4 ± Mustard		Meal 5 ± Wasabi	
Fixed meal	Foods	Serving size (g)	Foods	Serving size (g)	Foods	Serving size (g)	Foods	Serving size (g)	Foods	Serving size (g)
Pork Meat	Ham knuckle (Vastus lateralis) (S,F)	70	Diced, lean ham, outside muscle (M.biceps femoris) (S,F)	60	Pork loin, defatted (approx. 3 mm fat) (S,F)	108.8±1.7 (SE)	Filet Royal, lean Serono ham (S,F)	70 10	Shredded, lean ham, outside muscle (S,F)	70
Vegetables/ Fruit	Baked potatoes (W) Baked carrot (W) Baked celery root (W)	40 20 20	Beetroot (W) Apple (U)	80 40			Sugar peas (W) Potato (W)	40 20	Mango (W) Grape (B)	10 40
Salad	Cabbage lettuce (C) Iceberg lettuce (C) Rucula (B) Dried hazel nut (F,C) Pumpkin seeds (F,C)	10 20 10 5 5	Spinach (B) Iceberg lettuce © Feta cheese (F) Pine nuts (F,C)	20 20 10 5	Macaroni (W) Red capsicum (W) Broccoli (B) Raisins (W) Cashew nuts	50 20 50 10 10	Bulgur, Wheat (W) Cucumber (C) Parsley (C,B) Tomato (W) Lemon juice (U)	70 20 2 20 2.9	Avocado (F) Rice (W) Asparagus (C,B) Carrot (C,W) Shitake Mushroom Sea weed	5 52.3 5 5 1 2.2
Dressing	Frozen raspberry (U,W) Lemon juice (U) Honey (W) grape seed oil (F)	24.9 1.2 1.2 3.2	Yoghurt natural (U,F) Honey (W) Soya (S) Lemon juice (U)	27 3 2.6 0.1	Dried apricot (W) Lime juice (U) Orange juice (U) Grape seed oil (F) Balsamic vinegar (U)	11.4 2.1/5.7* 9.6/16* 3 3	Yogurt natural (U,F) Brown sugar (W) Elder flower vinegar (U) Grape seed oil (F) Dijon mustard (H) Coarse mustard (H)	7.2/26.4 2.4 0.96 7.1 9.6 9.6	Soya (S) Ketchup (W) Garlic (H) Hoisin sauce (W) Sugar (W) Vinegar (U)	22.1 2.1 1.1 2.1 1.7 17.4
Spices	Salt (S) Chilli pepper, fresh (H) Chilli powder	0.3 0.3 0.3	Salt (S) Horseradish (H)	0.5 10	Salt (S) Ginger (H)	0.5 20	Salt (S) Turmeric (B)	0.5 0.24	Wasabi	4
Energy %	Total amount Total energy Spicy Non-spicy Energy density (kJ/g) 30 % Protein 41 % Fat 29 % Carbohydrate	230.9 g 1042 kJ 1041 kJ 4.5	Spicy Non-spicy Total amount Total energy Spicy Non-spicy 31-32 % Protein 32-33 % Fat 35-37 % Carbohydrate	277.9 g 267.9 g 1040 kJ 990 kJ 3.9	Spicy Non-spicy Total amount Total energy Spicy Non-spicy 25 % Protein 42-43 % Fat 33-34 % Carbohydrate	301.5 g 295.3 g 2066 kJ 2025.8 kJ 6.9-7.0	Total amount Total energy Spicy Non-spicy 24 % Protein 20-22 % Fat 54-56 % Carbohydrate	292.5 g 2056.6 kJ 1986.5 kJ 6.8-7.0	Spicy Non-spicy Total amount Total energy 25 % Protein 11 % Fat 64 % Carbohydrate	319.6 g 315.6 g 1494.7 kJ 4.7
Buffet	Pork Vegetables Salad Nuts Dressing± chilli	50 40 40 10 30	Pork Vegetables Salad Feta cheese Nuts Dressing Horseradish	50 60 20 10 5 15 10	Pork Vegetables Salad Nuts Dressing ± Ginger	57.4±1.1 68.1 10 25/20 *	Pork Sugar peas Bulgur salad Potato with ham Dressing ± Mustard	50 20 61 24.5 15	Pork Sushi rolls ± wasabi Temaki rolls ± wasabi Grape Mango Soya Carrot, asparagus, avocado	53.75 86±2 124±2 20 10 20 5 g each

Paper IV

Effects of Capsaicin, Green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance



Paper IV

Supplementary theory

Energy expenditure

Energy for BMR is mainly used for maintaining sleeping metabolic rate (SMR), and a minor part is used for the cost of arousal (5-10 % of daily EE) ^{38,245}. BMR is closely related to body size and composition, why clear individual differences are observed and thus BMR is often predicted as a linear function of fat-free mass (FFM), one of the main determinant of BMR, or from height, weight and age taking gender into account ^{1,15,246-252}. In the following work we used the classical equations by Harris and Benedict (1919) to calculate BMR (MJ/day):

Female: $2.74 + 0.774 H + 0.040 BM - 0.02 A$

Male: $0.28 + 2.093 H + 0.058 BM - 0.028 A$

H = height (m), BM = Body mass (kg) A = Age (years).

Apart from BMR, physical activity and DIT (5-15 % of daily energy expenditure) also contribute to the daily energy expenditure ^{1,38,246,253,254}. DIT is the increase in energy expenditure observed after ingestion of a meal, which is regulated via the SNS. The energy expended for DIT is used for digestion, absorption and storage of nutrients and is largely dependent on the macronutrient composition and energy content of the food ^{11,38,255-258}. The highest and most prolonged DIT is obtained for proteins followed by carbohydrate and least for fat and several studies have conformed that high protein diets result in greater increase in DIT than when compared to a low protein diet, high fat or high carbohydrate diet ^{63,259-264}. EE is mainly determined by FFM however other factors such as physical activity, gender, genetic background, sleeping and aging do also have significant impact on EE ^{1,15,38,251,252,259,265-272}.

Energy balance

When energy intake equals energy expenditure humans are in energy balance with a stable body weight ¹. However during short term overfeeding with weight gain energy expenditure and fat oxidation will increase as a compensatory mechanism whereas underfeeding with weight loss will decrease energy expenditure and increase fat oxidation ^{18,273-275}. The compensatory decrease in energy expenditure during weight loss gives one possible explanation to why it is so difficult to maintain weight loss.

Effects of Capsaicin, Green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance

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Abstract

Background: Capsaicin, catechins, caffeine and CH-19 sweet pepper have been shown to stimulate energy expenditure and fat oxidation and to some extent reduce appetite and energy intake. The magnitude of these effects might depend on energy balance.

Objectives: To investigate how capsaicin, green tea, CH-19 sweet pepper as well as green tea and capsaicin added to three daily meals during respectively negative and positive energy balance affect appetite and energy intake.

Design: 27 subjects were randomized to three weeks of negative energy balance and three weeks of positive energy balance during which they received: 1) capsaicin, 2) green tea, 3) CH-19 sweet pepper 4) capsaicin + green tea and 5) placebo treatment. The effects on appetite (100 mm VAS), energy intake (kJ), body weight and heart rate were assessed.

Results: Positive or negative energy balance would affect possible treatment effects of bioactive ingredients on energy intake. CH-19 sweet pepper and a combination of capsaicin and green tea reduced energy intake only during positive energy balance ($p < 0.05$).

Capsaicin and green tea suppressed hunger more and increased satiety more during negative than during positive energy balance ($p < 0.05$).

Discussion: This implies that that bioactive ingredients may be helpful in reducing energy intake to prevent body-weight gain, and support body-weight loss periods of time by relatively sustaining satiety and depressing hunger.

Conclusion: Thermogenic food ingredients had energy intake reducing effects when used in combinations, and in positive energy balance.

Energy balance did not affect possible treatment induced energy intake, but did affect aspects of appetite profile, thereby supporting negative energy balance.

Keywords: capsaicin, caffeine, tea catechins, energy intake, energy balance, appetite, satiety, humans

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Introduction

Although weight loss approaches using herbal agents such as green tea or capsaicin have recently gained more attention due to the beneficial effect on the targets in body weight regulation, namely satiety, energy-metabolism, substrate oxidation and body composition (1-6) little attention has been paid to effects on appetite and energy intake (7;8). Increased activity of the sympathetic nervous system (SNS) after ingestion of capsaicin suggests that the reduction in energy intake could be due to the anorectic effect of catecholamines (8). Largest reduction in energy intake was observed when capsaicin was administered orally compared to gastrointestinal stimulation (7;9) suggesting that sensory stimulation of capsaicin is of importance to the total response (7). Since using capsaicin over a longer period of time is hardly possible due to bad compliance (7;10), the non-pungent CH-19 sweet pepper (*Capsicum annuum* L.) could be an attractive alternative to capsaicin. Not only a single dose of CH-19 sweet pepper increased body temperature and oxygen consumption (11), also repeated CH-19 sweet pepper intake for two weeks reduced body weight and enhanced fat oxidation by increasing the SNS in humans (12). However little is known about how CH-19 sweet peppers affect energy intake and appetite.

Two active compounds, catechins and caffeine, contribute to the beneficial metabolic effects of green tea (13-20). Few studies have looked at the effect of green tea on energy intake and appetite however Kao et al (16) showed that epigallocatechin-3-gallate (EGCG) from green tea reduced food intake and body weight in rats within 7 days and that the effects of EGCG were dose-dependent.

In both animals (21-23) and humans (1;24-26) positive energy balance increases activity of the SNS supporting that SNS has a role in counteracting positive energy balance to maintain energy balance (1). Taking these findings into account we hypothesized that possible effects of hot spices and green tea reducing energy intake might depend on energy balance.

The aim of this study was to investigate how herbal supplements of 1) capsaicin 2) green tea, 3) CH-19 sweet pepper as well as 4) capsaicin and green tea added to three daily meals during respectively negative and positive energy balance affect appetite and energy intake. In order to find evidence for this concept this study started to assess the hypothesis in normal weight subjects.

Subjects and Methods

Subjects

27 healthy subjects (10 men and 17 women) with a mean age of 26.9 ± 6.3 years (mean \pm SD), mean BMI of 22.2 ± 2.7 kg/m² and without any food allergies were recruited from the population in and around Maastricht University and hospital. The study was approved by the medical ethics committee of the Maastricht University Hospital. A power calculation based

upon Flint et al. (27) showed that with an effect size of 10 % and a 90 % power, a sample size of 24 subjects would be needed to detect 10 mm difference in the Appetite VAS. With an expected drop out of 10 % final sample size was determined to be 27 subjects. The height and weight of the participants were measured to calculate body mass index (BMI) and attitude towards eating: cognitive restrained and unrestrained eating behavior (F1), emotional eating and disinhibition (F2) and subjective feeling of hunger (F3) were assessed by using a validated Dutch translation of 'The Three-Factor Eating Questionnaire' (28). All subjects participated in the study for six weeks and were randomly assigned to three weeks of positive energy balance and three weeks of negative energy balance.

Procedure

Each subject participated in a six weeks crossover meal study where ten test meals, were consumed under two different conditions, respectively three weeks of negative energy balance and three weeks of positive energy balance. Half of the participants (half men half women) were randomly assigned to negative energy balance during the three first weeks and the other half to positive energy balance and after three weeks all participants shifted conditions. No test meals took place during one week after the shift of energy balance conditions. Serving orders of the treatments were randomized and balanced by means of Hall test calculations (29). On test days participants wore a heart rate monitor (Polar RS400, Finland/USA) all day; they were weighed before breakfast, lunch and dinner on a digital scale accurate to 0.02 g (Chyo-MW-150 K, Japan) and scored appetite on 100 mm visual analog scale (VAS) before, in between and after every meal, a total of eight scores a day.

Energy balance

During the six weeks subjects came fasted in the morning and were served standardized breakfast and lunch from Monday till Friday, at the University laboratory eating room. Food intake during the evenings and weekends was prepared and consumed by the subjects, following their own habits.

When subjects were fed to positive energy balance they received 20% of their individually calculated daily energy requirement for breakfast and 40 % of their individually calculated total daily energy requirement for lunch. When subjects were fed to negative energy balance 10 % of their individually calculated total daily energy requirement was provided for breakfast and 15 % of their individually calculated total daily energy requirement was provided for lunch. They had to consume all the food provided. Normally, 15 % of total energy requirement is given for breakfast, and 30-35% for lunch, so with these provisions it was feasible to reach positive and negative energy balance during 3 weeks. Estimates of subject specific daily energy intake were made by calculating basal metabolic rate (BMR) from the Harris-Benedict equation and multiplying BMR with a personal physical activity index (PAI) of 1.5-1.9, based upon the

outcome of the Baecke questionnaire (30).

The standardized breakfast consisted of orange juice, low fat strawberry quark and typical Dutch spicy breakfast cake (table 1). At lunch time participants got a baguette with cheese and ham, a baguette with apple syrup, a raisin roll and an apple during the negative energy balance condition. During the positive energy balance condition participants got larger baguettes, more raisin rolls, an apple and a pear. The energy

content of the meals were calculated from the nutrition information on the food items or from the standard Dutch NEVO food composition table (31).

Being in positive or negative energy balance was to be confirmed by the ultimate weight changes after each of the three weeks.

Table 1 Composition, serving size (g) and energy content (kJ) of the meals based on a energy requirement of 10 MJ per day

	Food	Negative energy balance 10 % Breakfast 15 % Lunch			Positive energy balance 20 % Breakfast 40 % Lunch		
		g	Kj	Energy content	g	Kj	Energy content
Breakfast	Orange Juice	163	260.8	Protein 17 %	333	532.8	Protein 17 %
	Spiced breakfast cake	39	503.1	Fat 3.5 %	76	980.4	Fat 3.5 %
	Kvark yoghurt	163	244.5	Carbohydrates 79.5 %	333	499.5	Carbohydrates 79.5 %
	Total	365	1008.4		742	2012.7	
Lunch	Multigrain brown baguette	68	639	Protein 15%	201	1889.4	Protein 17%
	Ham	6	28.8	Fat 8 %	26	124.8	Fat 10.5 %
	Cheese	6	94.2	Carbohydrates 70 %	26	408.2	Carbohydrates 65,5 %
	Apple syrup	2	23.4		16	187.2	
	Raisin roll	38,6	413.0		77,2	826.0	
	Apple	150	310.5		150	310.5	
	Pear	-	-		130	261.3	
	Total	270.6	1509.1		626.2	4007.44	
Ad libitum dinner	Lasagne	900-1800	4500-9000		900-1800	4500-9000	
	Salad with oil/vinegar	310	247		310	247	

^a

Test days

Each subject participated in ten test days during the intervention period where the treatments, capsaicin, green tea, CH-19 sweet pepper, capsaicin + green tea and placebo were ingested with the subject specific breakfast and lunch in the ongoing energy balance condition, and with an ad libitum dinner. Subject had two test days each week on non-consecutive days, and one week without testdays after energy balance conditions were shifted, to avoid carry-over effects from one treatment to the other. The test meals were consumed individually in the breakfast-, lunch-, and dining-rooms adjacent to the university laboratory kitchen.

The following 5 different treatments were applied randomly: 1) capsaicin capsules (510 mg cayenne, 40.000 Scoville heat units (SHU)), 2) 3.5 dl green tea drink (598.5 mg catechins, 77 mg caffeine), 3) CH-19 sweet pepper capsules (2.3 mg capsiate, Ajinomoto, Japan), 4) 3.5 dl green tea drink (598.5 mg catechins, 77 mg caffeine) + capsaicin capsules (40.000

SHU) and 5) placebo capsules with every meal. In addition to capsules (capsaicin, CH-19 sweet pepper and placebo) subjects also received 3.5 dl of water to assure that the same amount of liquid was consumed on every test day. Thus the total amount of active compound consumed on a test day was 7mg capsiate for CH-19 sweet pepper. The green tea was imported from Kao, Japan and contained the active compounds catechins (galliccatechin (GC), epigallocatechin (EGC), catechin (C), epicatechin (EG), epigallocatechine gallate (EGCG), galliccatechine gallate (GCG), epicatechine gallate (ECG), catechine gallate (CG)) and caffeine providing a total of 1795.5 mg catechins and 231 mg caffeine on every test day. In this paper, this mixture of tea catechins and caffeine is referred to as 'green tea'.

For the ad libitum dinner meal participants received on average 1.5 kg of Italian lasagne and 300 g of a mixed salad with 10 g of an oil/vinegar dressing (Table 1). Serving size was as such that always leftovers remained on the plate. Total ad libitum food intake was measured by weighing the food before and after serving.

Ratings of Appetite, liking and food desires with the test meals

Appetitive ratings were made eight times a day, before and after every meal, in between breakfast and lunch and between lunch and dinner. Liking was anchored with 'not at all' and 'very much' and scored before and after breakfast, lunch and dinner. Furthermore participants scored their desires to eat sour, sweet, fat, bitter, salty and hot stimuli before and after every meal, in between breakfast and lunch and between lunch and dinner ranging from 'not at all' to 'very much'.

All ratings were scored on 100 mm VAS. Participants were asked to score their appetite as *hunger* ranging from 'not at all hungry' at 0 till 'very hungry' at 100 mm, *desire to eat* from 'very weak' till 'very strong', *prospective food consumption* from 'nothing at all' till 'a very large portion', *fullness* from 'not at all full' till 'very full' and finally *satiety* was scored from 'not at all satiated' till 'very satiated'.

Data analysis/Statistical analysis

The values of all test parameters are presented as the mean±standard error (SE). Results were expressed either in actual mean values or values of treatment minus placebo (Δ values).

The repeated measures of weight (before breakfast, lunch and dinner) were analyzed by linear mixed statistical models (proc mixed) in SAS (SAS Institute, Inc., Cary, NC, USA) and evaluated for significant effects of time of the day, conditions :positive/negative energy balances. A mixed repeated analysis was made on appetite measures (random effect: subject; fixed factor: treatment, gender, time and condition; covariates: BMI, age, baseline appetite, temperature of the test day and three-factor eating scores (factor 1, factor 2 and factor 3)) as well as desires to eat sour, sweet, fatty, bitter, salty and hot scores (random effects: subject, test day; fixed factor: treatment, gender, time of the day, desire and condition; covariates: BMI and age) and liking scores (random effects: subject, test day; fixed factor: treatment, gender, time of the day and condition; covariates: BMI, age, temperature of the test day and three-factor eating scores (factor 1, factor 2 and factor 3)). In addition mixed models were used to analyze the ad libitum energy intake (kJ and g) (random effects: test day, subjects; fixed factor: gender, time of dinner, condition and treatment; covariates: BMI, age, temperature, liking before the meal, hunger and factor 1, factor 2 and factor 3) and the heart rate measures (random effects: subject, test day; fixed factor: gender, condition and treatment, covariates: BMI, age and temperature). Significance was set at a p-value <0.05. When statistically significant differences were detected, a post hoc pairwise comparison across treatments and conditions was performed by using Tukey-Kramer's test and the corresponding adjusted p-value (adj p) was found.

Results

Subject characteristics are summarized in Table 2

Table 2 Subject characteristics (number or mean± standard deviation (SD))

	Women	Men	All
N	17	10	27
Age (years)	23.6±2.6	32.5±7.0	26.9±6.3
Body weight (kg)	62.4±6.6	75.8±15.0	67.3±12.2
BMI (kg/m ²)	21.7±1.7	23.0±3.8	22.2±2.7
PAI	1.7±0.1	1.7±0.1	1.7±0.1
ADMR (MJ/24 hr)	11.3±1.1	12.9±1.9	11.9±1.6
Restraint (Factor 1)	8.5±4.5	5.2±3.9	7.3±4.5
Disinhibition (Factor 2)	5.9±3.4	4.4±2.9	5.4±3.2
Hunger (Factor 3)	5.8±2.8	3.9±3.6	5.1±3.2

PAI:physical activity index; ADMR: average daily metabolic rate; Restraint, disinhibition, hunger are scores of the Three factor eating questionnaire by Stunkard and Messick, 1985.

Positive and negative energy balance

In general, differences between positive and negative energy balances were observed with respect to body weight, heart rate, ad lib energy intake, appetite and liking of the food.

Body weight

Body weight changes during the three weeks of negative energy balance and three weeks of positive energy balance (Δ Body weight) were calculated by subtracting the baseline body weight (body weight measured respectively before the three weeks of negative energy balance and before the three weeks of positive energy balance) from the body weights measured on the test days. A significant condition* testday interaction ($p<0.01$) revealed that subjects gained weight during the positive energy balance period and lost weight during the negative energy balance period. During the positive energy balance period body weight was increased by 0.50 ± 0.2 kg ($p<0.05$) whereas body weight was decreased by 0.44 ± 0.2 kg ($p<0.05$) during negative energy balance period. So the conditions of positive and negative energy balance, which were aimed for, were achieved.

Heart rate

Similarly, pairwise comparisons of the effect of condition showed that heart rate was significantly higher during positive energy balance when compared to negative energy balance. Heart rate during positive energy balance condition, measured

on the day when placebo was offered, was increased by 4.1 ± 1.4 beats/min ($p < 0.01$) compared to heart rate during negative energy balance condition.

Ad lib energy intake

A third general difference between effects of positive and negative energy balance was energy intake during dinner. The overall significant condition effect ($p < 0.01$) revealed that subjects ate significantly more of the ad libitum dinner during the negative energy balance than during the positive energy balance.

Appetite

Moreover, positive and negative energy balances were proven to affect appetite. Positive energy balance led to significantly higher sensation of fullness and satiety as well as significantly lower feelings of hunger, desire to eat and prospective food consumption ($p < 0.0001$; data not shown).

Also desire for eating sour, sweet, fatty, bitter, salty and hot foods were significantly higher during the negative energy balance than during the positive energy balance (adj $p < 0.0001$).

Furthermore liking of the meals was significantly higher ($p < 0.01$) during negative energy balance when compared to positive energy balance (data not shown). However the decrease in liking over a meal was significant during breakfast, lunch and dinner, during both negative and positive energy balance ($p < 0.05$, data not shown)

Effects of capsaicin, green tea, CH-19 sweet pepper and capsaicin + green tea on energy intake, appetite, liking, and heart rate.

To test the hypothesis, that the effects of treatments differ between positive and negative energy balance, pairwise comparison between treatments and placebo were made for the two conditions, positive and negative energy balance (table 3). For treatment * condition interaction see adjusted p-values (table 3).

No significant interactions affecting energy intake were shown. Effects on energy intake were present as a significant decrease in energy intake during positive energy balance with the treatments: CH-19 sweet pepper, and capsaicin+green tea (table 3).

With respect to appetite scores (hunger, desire to eat, feeling full, satiety) significant interactions were shown. These appetite ratings were all affected by the treatment green tea+capsaicin, yet stronger in negative than in positive energy balance (table 3).

Furthermore, incidentally differences in some aspects of appetite were shown, yet no convincing multiple significances were present.

Furthermore the combination of capsaicin and green tea significantly reduced liking over the meals ($p < 0.0001$, adj. $p < 0.0001$) when compared to placebo whereas green tea, capsaicin and CH-19 sweet pepper individually did not affect liking over the meals suggesting that combining bioactive ingredients contribute to a stronger sensory specific satiety (SSS) over a meal.

A significant treatment*desire interaction ($p < 0.0001$) revealed that capsaicin, green tea and the combination of capsaicin and green tea significantly reduced desire to eat fatty, salty and hot ($p < 0.05$) whereas CH-19 sweet pepper did not affect any desires. Furthermore capsaicin reduced desire to eat sour ($p < 0.05$) and the combination of capsaicin and green tea reduced desire to eat bitter ($p < 0.05$). These desires were significantly reduced by treatments during both positive ($p < 0.05$) and negative energy balance ($p < 0.01$).

Apart from the effect of positive energy balance on heart rate, no additional effects on average daily heart rate were shown during administration of capsaicin, green tea, CH-19 sweet pepper and the combination of capsaicin and green tea.

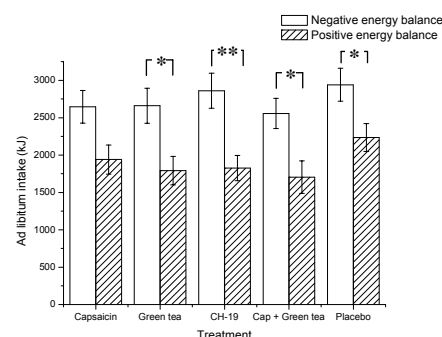


Figure 1 ad lib energy intake (Mean values \pm SE) for each of the five treatments during negative and positive energy balance. Notation of significances * (5 %), ** (1%) and *** (0.1%) specify significant effects of energy balance on ad lib energy intake for each treatment.

Table 3 Estimated values of the treatment effects (treatment – placebo) calculated from the statistical model (Differences of LSMeans \pm SE).

	Ener gy bala nce	Ad libitum intake (kJ)	p- value / Adj p- value	Hunger (mm)	p-/ Adj p- value	Desire to eat (mm)	p- value/ Adj p- value	Full (mm)	p- value/ Adj p- value	Satiety (mm)	p- value/ Adj p- value	Av. heart rate (beats/m in)	p- value
Capsaicin	Neg.	-304.4 \pm 162.7	ns	-3.4 \pm 2.3	ns	-1.5 \pm 2.4	ns	7.0 \pm 2.6	0.007	5.4 \pm 2.7	0.043	-0.3 \pm 1.4	ns
	Pos.	-181.6 \pm 161.5	ns	-5.2 \pm 2.3	0.023 ns	-4.7 \pm 2.4	ns	2.0 \pm 2.6	ns	3.3 \pm 2.6	ns	-1.3 \pm 1.4	ns
Green tea	Neg.	-277.6 \pm 162.8	ns	1.8 \pm 2.3	ns	1.2 \pm 2.4	ns	5.8 \pm 2.6	0.028 ns	3.9 \pm 2.7	ns	0 \pm 1.4	ns
	Pos.	-310.0 \pm 163.1	ns	-4.9 \pm 2.3	0.035 ns	-4.5 \pm 2.4	ns	2.5 \pm 2.6	ns	4.8 \pm 2.7	ns	-0.9 \pm 1.4	ns
CH-19 sweet pepper	Neg.	-215.87 \pm 164.8	ns	3.8 \pm 2.3	ns	2.9 \pm 2.4	ns	1.2 \pm 2.6	ns	-0.3 \pm 2.6	ns	-1.4 \pm 1.4	ns
	Pos.	-360.34 \pm 162.2	0.027 0.445	-3.3 \pm 2.3	ns	-2.7 \pm 2.4	ns	0.1 \pm 2.6	ns	0.9 \pm 2.7	ns	-2.3 \pm 1.4	ns
Capsaicin + green Tea	Neg.	-307.42 \pm 162.4	Ns	-8.1 \pm 2.3	<0.001 0.018	-9.1 \pm 2.4	<0.001 0.007	12.7 \pm 2.6	<0.001 <0.001	13.4 \pm 2.6	<0.001 <0.001	-0.6 \pm 1.4	ns
	Pos.	-370.33 \pm 161.8	0.023 0.400	-5.5 \pm 2.3	0.018 ns	-5.1 \pm 2.4	0.036 ns	5.5 \pm 2.6	0.035 ns	5.7 \pm 2.7	0.033 0.499	-1.4 \pm 1.4	ns

*p-values indicate significant difference between the treatment and placebo for respectively negative energy balance and positive energy balance. mm = mm VAS, LSMeans, Least Squared Means; Differences of LSMeans are calculated as LSMeans for Treatments minus LSMeans for placebo. Thus negative values for differences in LSMeans indicate that the treatment has reducing effect whereas positive values in the table indicate enhancing effect of the treatment when compared to placebo.

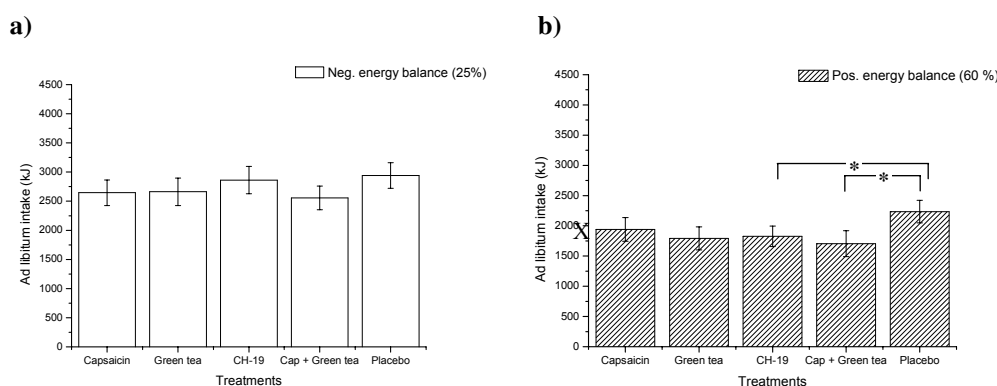


Figure 2 ad lib intake (Mean values \pm SE) during a) negative energy balance and b) positive energy balance. Notation of significances * (5 %), ** (1%) and *** (0.1%) specify significant effects of treatments on ad lib intake.

Discussion

The present study did not support our hypothesis that positive

or negative energy balance would affect possible treatment effects of bioactive ingredients, namely capsaicin, green tea, CH-19 sweet pepper, and a combination of green tea and capsaicin on energy intake.

Being in negative or positive energy balance was confirmed by losing or gaining body-weight. Further characteristics of negative versus positive energy balance were a lower heart rate, a higher *ad lib* energy intake, appetite, and liking of the food.

Interaction between energy balance and appetite profile aspects (hunger, desire to eat, fullness or satiety) was shown as hunger and desire to eat were more reduced, while fullness and satiety were more increased during negative energy balance than positive energy balance for the capsaicin plus green tea treatment. Obviously, the strongest treatment, capsaicin plus green tea induced greater satiety and diminished hunger, during negative energy balance. This phenomenon meets one of the conditions for weight loss during negative energy balance, namely sustained satiety, and prevention of increases in hunger.

Although these appetite profiles contributed to reducing energy intake, the magnitude of reduction did not reach a significant effect of energy balance. Only, in positive energy balance a significant reduction of energy intake following capsaicin and green tea consumption was shown, as well as following CH-19 sweet pepper administration.

Since the strongest treatment showed most significant effects, it seems that synergism of bioactive ingredients is of importance.

Previously it has been shown that a combination of bioactive ingredients has a stronger energy intake reducing effect than treatment with single ingredients. Toubro et al (32) found that body weight loss was greater with a combination of caffeine and ephedrine whereas Dulloo et al. (13) showed that stimulating effects of green tea on energy expenditure and substrate oxidation due to caffeine and tea catechins were greater than explained by the active compounds given individually. More recent studies have focused on the effects of bioactive ingredients given in combination and found energy intake or appetite reducing effects in the short (33;34) and in the long term (4;5;35-37).

These results support our findings that capsaicin in combination with green tea can induce considerable changes in energy intake. Since the non-pungent CH-19 sweet pepper was an efficient suppressor of energy intake as well it would be of interest to investigate if a combination of CH-19 sweet pepper and green tea leads to a similar synergistic effect on energy intake. Liking over the meals supplemented with capsaicin plus green tea was significantly reduced suggesting that the combination of capsaicin and green tea contributed to stronger SSS, which might lead to a smaller energy intake as well.

In summary, this study does not support the hypothesis that positive or negative energy balance would affect possible treatment effects of bioactive ingredients on energy intake.

CH-19 sweet pepper and a combination of capsaicin and green tea reduced energy intake only during positive energy balance. Furthermore capsaicin and green tea suppressed hunger more

and increased satiety more during negative energy balance.

Effects of green tea and capsaicin were partly synergistically, while sensory effects contributed to the observations.

Implications of these results are that bioactive ingredients (capsaicin, green tea, CH-19) may be helpful in reducing energy intake to prevent body-weight gain. At the same time these bioactive ingredients may support body-weight loss periods of time by relatively sustaining satiety and depressing hunger.

We conclude that thermogenic food ingredients had energy intake reducing effects when used in combinations, and in positive energy balance. Energy balance did not affect possible treatment induced energy intake, but did affect aspects of appetite profile, thereby supporting negative energy balance.

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Summary of Results

In Part I focusing on ‘Sensory Properties of Spicy Meals’, our results indicate that components in spicy meals do interact and that chili can suppress flavour in solid foods and that the masking of flavour is dependent on the capsaicin concentration (table 8.1, paper I). Masking of flavour by capsaicin could occur via a central neural integration, however a preliminary study suggests that interaction between the gustatory and the trigeminal sense could occur on the peripheral level since capsaicin might suppress taste cell signalling in animals (table 8.1, manuscript 1). Since the literature on texture and its relations to taste and flavour perception is scarce, we found it of interest to look at how texture in solid foods affects oral burn. The two textures that were tested had no significant effects on flavour or oral burn (table 8.1, paper I). Furthermore oral burn has been shown to increase linearly with temperature suggesting that pain receptors and heat receptors interact. We found a non-linear temperature dependency of oral burn, since pork patties served at 38 °C were slightly more burning than at 67 °C and least burning at 8 °C (table 8.1, paper II). Additionally minor findings indicate that oral burn is significantly more persistent for non-eaters of chili than for eaters of chili (table 8.1, paper I).

In part II ‘Spicy Meals, Appetite and Energy intake’ it was investigated if bioactive ingredients affect appetite and energy intake. Adding tolerable doses of hot spices to a fixed meal had no evident effects on appetite and energy intake at a succeeding buffet (table 8.2, paper III). Additionally minor findings indicated that hot spices affect sensory specific desires (table 8.2, paper III). However, three daily exposures of bioactive ingredients revealed that CH-19 sweet pepper and the combination of green tea and capsaicin reduce energy intake under conditions of positive energy balance whereas capsaicin and green tea reduced appetite during negative energy balance (table 8.2, paper IV).

Table 8.1 Overview of the results obtained in part I ‘Sensory Properties of Spicy Meals’

Study	Subjects	Study design	Effects on sensory properties of spicy food	Mechanisms/conclusions
Paper I ²⁷⁶	8 professional sensory panellists	Food 6 Pork patties with two textures (0.5 and 5 w/w % flour) ± chilli powder or minced chilli (1 w/w %) Method TI, ISI= 25 min., HPLC Attributes Oral burn, meat flavour	Chilli powder and minced chilli suppress Meat flavour No effect of The two textures (too similar or no effect?) Minced chilli < Oral burn < Chilli powder Minced chilli (capsaicin: 0.97 mg/g chilli) Chilli powder (capsaicin: 1.23 mg/g chilli) Individual differences for chilli burn assessment (eater/non-eater of chilli)	Capsaicin suppression could be a psychological effect (dominating chilli burn) or interaction between trigeminal, gustatory and olfactory sensations (due to either a central neural effect or peripheral effects) Prior experiences, chronic desensitization or difference in sensitivity might explain difference between eaters and non-eaters of chilli.
Paper II ²⁷⁷	2 professional sensory panellists + 8 trained students	Food 12 Pork patties with four chilli concentrations (0.25, 0.5, 1, 2 5 w/w % chilli powder) and three temperatures (8, 38 and 67 °C) Method TI, ISI= 30 min. Attributes Oral burn	Oral burn is increased by Chilli concentration Maximum intensity of the oral burn increased in a dose-dependent manner. Temperature (8°C < Oral burn < 38 = 67 °C) independently of capsaicin concentration	Temperature dependence of oral burn can be caused by interaction between heat receptors and the capsaicin binding TRPV1. It is hypothesized that binding of capsaicin to its TRPV1 might have optimum at 37°C (the temperature of the oral cavity). The effect of temperature on oral burn was independent of capsaicin concentration suggesting that the few temperature points or the taste complexity and texture of the pork patties may have reduced that impact of temperature on oral burn
Manuscript I	Rat (tissue slices) Pig (whole tissue)	Stimuli Tastants/capsaicin Method Immunostaining of ERK2-p/ERK2 in taste cells. Confocal laser scanning micrographs.	Sucrose > Sucrose + capsaicin activation Filiforms bend after capsaicin stimulation ERK1+2-p/ERK1+2 activity in filiform papillae, supporting cells, taste buds	Capsaicin might suppress sweet taste signalling in rat. Capsaicin might destroy epithelia in rat tongue or the cutting might have destroyed the tissue. The specificity of the assay may not be high enough to detect taste signalling. The assay only gives a snapshot of the cell activity.

ISI, Interstimulus interval; min., minute(s); TRPV1, Transient receptor Potential Vanilloid receptor 1; TI, Time-Intensity; W/W, weight/weight.

Table 8.2 Overview of the results obtained in part II ‘Spicy Meals, Appetite and Energy intake’

Study	Subjects	Study design	Effects on metabolism	Mechanisms/conclusions
Paper III 278	40 young healthy subjects 17 men 23 women	Meal Five different meals of fixed portions size \pm hot spices, ad libitum buffet Treatment Control, 0.6 g chilli, 10 g horseradish, 20 g ginger, 19.2 g mustard, 4 g wasabi. Condition free living	Energy intake Ginger reduced energy intake (only in men) Appetite Ginger reduced desire to eat (only in men) Wasabi increased prospective food consumption Chilli increased desire to eat in women Sensory specific desires Mustard reduced desire for salty Chilli increased desire for sweet No effect Chilli, horseradish, mustard and wasabi on energy intake Horseradish, mustard on appetite	Amount of food consumed was relatively constant and independent of energy content Hot spices might change desires and may affect food choice and energy intake Larger doses of hot spices as well as several exposures per day might be necessary to obtain appetite and energy suppression by hot spices
Paper IV 279	27 healthy subjects	Meal Five different meals of fixed portions size \pm hot spices, ad libitum buffet Treatment 1:capsaicin (515 mg cayenne, 40.000 SHU), 2:green tea (1795.5 mg catechins and 231 mg caffeine), 3: CH-19 sweet pepper (7 mg capsiate), 4:(capsaicin + green tea), 5:control Condition three wk. of negative EB, three wk. of positive EB	Energy intake was reduced by CH-19 sweet pepper, (capsaicin + green tea) during positive energy balance Appetite Capsaicin and green tea reduced hunger during positive EB and increased fullness during negative EB. Largest effects on appetite when combining capsaicin and green tea. No effect CH-19 sweet pepper on appetite All treatments on HR	Suppressive effects on energy intake were greater during positive than negative energy balance. Combinations of bioactive components exert greater suppressive effect on appetite during negative than positive energy balance. CH-19 sweet pepper might be a good alternative for the pungent chillies.

EB, energy balance; HR, heart rate; SHU, Scoville SP, sweet pepper; heat units; wk, week(s);

Discussion

As Winston Churchill once cleverly said 'However beautiful the strategy, you should occasionally look at the results' and by doing that I realized that not all of my initial ideas was turned into scientific achievements. The following discussion is divided into two parts, 'Sensory Properties of Spicy Meals' (part I) and 'Spicy Meals, Appetite and Energy intake (part II) with special emphasis on the four underlying research questions, which however were extended in accordance with the results obtained in the present PhD project (see italic sentences in figure 9.1).

Part I ‘Sensory Properties of Spicy Food’

1. How do components in spicy meals interact?

How does oral burn affect meat flavour in pork patties? (Paper I)

How does chilli concentration, serving temperature and texture affect oral burn in pork patties? (Paper I and II).

2. Which mechanisms can explain interactions between gustatory, olfactory and trigeminal stimuli?

- *Does capsaicin stimulate the bitter, sweet taste, signalling pathways in rat taste cells?(manuscript I)*
- *How does capsaicin affect pig tongue tissue?*

Part II ‘Spicy Meals, Appetite and Energy intake’

3. Do hot spices affect appetite and energy intake?

- *Does chili, horseradish, ginger, mustard and wasabi affect appetite and energy intake during a meal?(Paper III)*
- *Does capsaicin, green tea, CH-19 sweet pepper and a combination of capsaicin and green tea affect daily appetite and energy intake? (Paper IV)*

4. Which physiological mechanism can explain effects of hot spices on energy intake and appetite?

- *Do capsaicin, green tea, CH-19 sweet pepper and a combination of capsaicin and green tea affect daily heart rate (Paper IV)*



Figure 9.1

Part I Sensory Properties of Spicy Meals

1. How do components in spicy meals interact?

Hot spices such as chilli, horseradish, ginger, mustard, wasabi and black pepper are well-known stimulants of the trigeminal nerve. Furthermore capsaicin, zingerone and piperine, the pungent components in chilli, ginger and black pepper have been found to stimulate bitter taste^{116,124,125,159-163,280}. These findings suggest that irritants from hot spices possess both irritant and taste qualities or alternatively that trigeminal stimuli can interact with gustatory and olfactory stimuli. The fact that irritants possess taste qualities or that interactions between senses occur during food consumption could explain why hot spices enhance the overall flavours of food, which is the general belief^{124,125,164,165}. Several studies have shown that capsaicin can suppress tastes in both solutions and in foods; however capsaicin suppression was more evident for sweet taste than for the other basic tastes^{124,151,170-172}. Furthermore pre-rinsing with capsaicin suppressed olfactory sensations (lemon and celery odour) to similar extent as taste stimuli suggesting that masking of taste and odours could be exerted via a common mechanism. Few studies have, looked at the effects of capsaicin on flavours in more complex foods. Pre-stimulation with capsaicin did not influence flavours in fruit beverages (peach, pear, apricot and passion fruit) and only minor capsaicin suppression of flavours (orange and vanilla) or no effects (strawberry flavour) were found in capsaicin/flavour, mixtures^{170,171}. The finding that strawberry flavour, which has been strongly correlated to sweetness, was not affected by capsaicin could suggest that capsaicin suppression is not caused by confusion between taste (sweetness) and flavour¹⁷¹. We discovered that chilli powder and minced chilli suppressed meat flavour in chilli spiced pork patties, suggesting that capsaicin suppresses flavour in solid foods²⁷⁶. Based on the current knowledge we believe that suppression of meat flavour was mainly caused by interactions between gustatory, olfactory and trigeminal stimuli via either a central neural integration of sensory input or via peripheral effects^{170,171}. Since we used a professional sensory panel for evaluation of meat flavour and oral burn we believe that the psychological, cognitive phenomenon, where the dominating oral burn draws attention away from tastes and flavours, plays only a minor role in capsaicin suppression of meat flavour. Two different chilli products were used in the present study, chilli powder and minced chilli. Chilli powder was perceived as the most burning of the two due to higher concentrations of capsaicin

and dihydrocapsaicin. These findings suggest a capsaicin concentration-dependent masking of meat flavour; however, more studies are needed to support this suggestion. Furthermore, the larger surface of the chilli powder particles and better distribution in the pork patties might have made capsaicin easier accessible to its receptors and thereby more burning than minced chilli. We therefore hypothesized that coarse chilli particles might be perceived less hot than small particle, which might be beneficial since larger amounts of spices can be added before the product becomes too hot. However, further investigations are needed to understand how particle sizes of the chilli products affect oral burn.

Contrary to the studies discussed above, which investigated how capsaicin can suppress tastes and flavours, several other studies have tried to reveal how oral burn can be suppressed by other qualities such as temperature, tastes and flavours, since such knowledge can also provide greater insight into how interactions between trigeminal, gustatory and olfactory sensations occur. Sweet taste has been shown to decrease oral burn²⁸¹. Furthermore oral burn of equally spiced water and liquid food systems (cheese, starch, oils) was found to decrease with increasing complexity and/or fat level of the samples^{167,174,175}. Solubilisation of the lipophilic capsaicin by fat molecules could interfere with binding of capsaicin to its pain receptor, TRPV1 and thus reduce oral burn in liquid systems¹⁶⁷. These findings were however not consistent with more recent results showing that an increase in fat levels in chilli spiced chicken patties increased oral burn¹⁷⁶. The fact that fat might act as a carrier of capsaicin to its pain receptor in solid food system could explain why oral burn was increased in chicken patties and why discrepancies were found between the present and previous studies using liquid food systems. A chemical binding between capsaicin and fat or between capsaicin and other components in the food matrix could also explain the different effect of fat on oral burn. Binding complexes between casein in cheese and capsaicin has previously been suggested to reduce oral burn²⁸². If food with its different textures, tastes and many flavours might reduce oral burn due to complexity and textures we hypothesized that the texture of the food could play a crucial role for the perception of oral burn. However we failed to show that the texture of chilli spiced pork patties affected oral burn but since only two texture levels were used in the study we can not rule out that the two textures might have been too similar²⁷⁶. Few studies have shown that intensity and release of sweet and flavour compounds are suppressed with increasing complexity of the texture indicating that texture can modify both taste and flavour perception²⁸³⁻²⁸⁵. Therefore we find it of interest to

study further how different textures affect perception of tastes, flavours and irritants as well as how irritant affect these qualities and overall flavour of the food.

Temperature of the food and oral cavity also affects perception of tastes, certain flavours and textures as well as the intensity of oral burn^{286,287}. Generally, oral burn increases with increasing serving temperature in simple solutions whereas the effect of temperature becomes less evident when oral burn is assessed in mixed taste/irritant solutions and complex foods^{124,167,180}. We found chilli spiced pork patties served at 38 to be slightly more burning than at 67 °C whereas the burning sensation of the pork patties were reduced at 8 °C²⁷⁷. Furthermore the temperature dependency of oral burn in pork patties were independent of the capsaicin concentration unlike previously where the temperature dependence of oral burn has been shown to increase with increasing capsaicin concentrations in simple solutions^{180,277}. The fact that cooling down of the pork patties to 8 °C suppressed the oral burn suggests that nociceptors and cold receptors may interact and thereby decrease the sensation of oral burn¹⁸⁰. However contrary to expectation we found chilli spiced pork patties served at 38 to be slightly more burning than when served at 67 °C²⁷⁷. Prescott et al.¹²⁴ found no effect of temperature on oral burn in tomato soup served at 37 and 60 °C. Too few serving temperatures tested as well the taste, flavour and texture complexity of the food may have reduced the impact of temperature on oral burn in foods^{124,167,277}. Alternatively, we hypothesized that the equal burn experienced in pork patties and tomato soup at different serving temperatures could be caused by a temperature-dependent binding of capsaicin to TRPV1 with optimum around the oral cavity temperature (37 °C). We believe that optimal binding at 37 °C might exert burning sensation similar to the ones experienced at higher temperatures, at which oral burn is intensified due to activating of TRPV1 and other heat nociceptors^{124,138,139,150,288-291}. Serving temperatures have previously been suggested to affect the rate at which the oral burn develops and the duration of the oral burn sensation^{167,180}. Since the rate might be controlled by how capsaicin binds to TRPV1 we think that this supports our theory of temperature dependent binding of capsaicin to TRPV1.

In summary previous and recent studies confirm that it is a challenge to study how oral burn from chilli is affected by temperature, texture, tastes and flavours as well as how chilli affects these qualities in real food. Even though we are far from a complete understanding of how irritants affect the overall flavour of food, the current research indicates that interactions between irritants, tastes and flavours do

occur and have some impact on the overall flavour of real foods. Sensory analyses using simultaneous evaluation of several attributes, or methods where the sensory properties of foods can be evaluated as they develop, have previously been described, though they are not widely used due to the complexity of the evaluation task ^{224,225,292-294}. However using such methods might provide better insight into how irritants, tastes and flavours, as well as different textures, interact in foods and how they influence sensory perception. Especially it would be interesting to clarify how fat concentration and texture affect oral burn and finally how irritants affect overall flavour of foods. Such effects could be studied in different food matrices such as liquid foods (meat gravy), semi-liquid (cream soup), solid foods (pork patties) and whole meals to investigate how oral burn and flavour develop in different carriers with varying texture and complexity of the food product.

2. Which mechanisms can explain interaction between gustatory, olfactory and trigeminal stimuli?

The fact that some hot spices are believed both to enhance and suppress tastes and flavours, as well as stimulate bitter taste, makes it challenging to propose a common mechanism for interaction between gustatory, olfactory and trigeminal stimuli during food perception ^{116,124,125,159-163,280}. Most theories suggest that interactions between gustatory, olfactory and trigeminal stimuli occur via a central neural integration of sensory input and/or via peripheral effects ^{123,124,170-173,179-181}. Several animal studies have provided evidence for a common neural mechanism between taste and irritation. Firstly, capsaicin has been shown to stimulate primary gustatory neurons of the chorda tympani and thereby suppress neural taste responses ^{181,239,295}. Secondly trigeminal afferents projected to NTS suppressed responses to taste in NTS gustatory neurons of the chorda tympani via substance P ²³⁸. The fact that capsaicin suppresses some tastes better than others might be caused by varying sensitivity of gustatory neurons and varying degrees of interaction ¹²⁴. Capsaicin significantly suppressed responses of gustatory NTS neurons in both intact and trigeminal ganglionectomized rats, suggesting that the suppression is not mediated by a central trigeminal effect. These findings suggest that taste and irritation act synergistically, which could be caused by taste having irritant properties, capsaicin having taste properties or that taste nerves might transmit irritation at the expense of gustatory signals ^{124,161-163,173,181,296}.

Peripheral interactions between irritation and taste have also been identified, showing that substance P released from trigeminal fibres can innervate taste buds and alter sensitivity of taste cells whereas capsaicin can inhibit activity of ion channels in both trigeminal nerve cells and taste cells ^{172,296-300}. Furthermore, capsaicin can provoke plasma extravasation and oedema in fungiform papillae ^{181,240}. Studies of the mechanism for capsaicin suppression of sweet taste revealed that capsaicin mediate suppressive effects through a TRPV1-independent mechanism affecting activity in taste cells ²⁴³. These findings might suggest that capsaicin can react directly with the taste receptors or taste cells to alter gustatory responses and furthermore be able to block the pore of taste buds in taste papillae by causing contractile responses ^{161,170-172,179,240}. To support this hypothesis we studied sweet and bitter signalling, measured as the ratio between ERK1-2 protein and its phosphorylated form, in the presence of taste (quinine or sucrose) and in taste/capsaicin mixtures in rat tongue tissue (manuscripts I). Our results indicated that capsaicin could suppress sweet taste signalling. However since both signal transduction pathways in taste cells and possible interactions with the trigeminal sense are still poorly understood, we believe that the present cellular assay in a more developed and optimised form could reveal if interactions between gustation and the trigeminal sense occur via direct or indirect activation of taste cells. Especially, a more targeted staining of important taste and irritant signalling mediators such as cAMP, Phospho-lipase C and gustducin as well as immunostaining of taste and irritant receptors, could provide a snapshot of insight into how taste and pungency are transmitted on the cellular level.

Finally, it can not be ruled out that capsaicin compete with taste and flavour stimuli on a central neural attentional level where the most dominant and powerful stimuli in foods suppresses the other qualities ^{124,169,171}. In accordance with previous findings we observed that eaters of chilli perceived chilli burn as less strong than non-eaters of chilli ²⁷⁶. Learning from previous experiences with oral burn as well as genetic differences in sensitivity to oral burn and susceptibility to evoke desensitization by capsaicin might explain why eaters of chilli experience oral burn less intensely than non-eaters of chilli ^{117,169-171,178}. However, a better understanding of how cognitive phenomena, sensitization/desensitization or genetic differences between assessors influence oral burn, taste and flavour perception is needed.

In summary, interactions between gustatory and trigeminal stimulation seem to involve both central neural and peripheral interactions. However, it is evident that

the previous and current research has focused on investigating how irritants and tastes interact whereas hardly any research has tried to elucidate mechanisms explaining how irritants interact with complex flavours and textures in foods. Flavours, tastes and irritants, as well as thermal and mechanical stimuli, may interact in more complex ways than presently thought, to produce overall flavour. Both contextual effects and different levels of interaction between oral, olfactory and trigeminal input in the nervous system should be considered in order to explain the mechanisms behind taste and flavour masking by capsaicin, difference in subjective sensitivity towards capsaicin and development of overall flavour. On the peripheral level, studies of chemical interactions between irritants, tastes and flavours and their receptors could reveal if and how these component interact, if they combine into chemical complexes and how such binding affect oral burn and total flavour perception of food. More research is needed to fully understand the basic characteristics of pungency¹¹⁶ and reveal if irritants can stimulate taste cells and thereby alter gustatory responses. Basic research could also involve studies investigating how individual sensitivity towards oral burn, difference in susceptibility to sensitisation and desensitization and sensitivity towards taste and flavour, as well as habituation to hot spices, affect the total flavour experience

^{112,116,125,163,171,173}.

Part II Spicy Meals, Appetite and Energy intake

3. Do hot spices affect appetite and energy intake?

Obesity develops due to long-term imbalance between energy intake and energy expenditure where sedentary lifestyle, increased accessibility and consumption of high energy dense food combined with genetic predisposition are believed to be the major contributors to the epidemic increase in obesity ^{1,4,37}. The great challenge for researchers is to find long-term strategies that can prevent or treat overweight and obesity in both children and adults. Commonly used weight loss approaches have focused on behavioural strategies, such as restricting the diet and increasing physical activity whereas others have tried to identify pharmaceutical or herbal supplements with beneficial effects on satiety, energy expenditure, substrate oxidation, body weight and composition. Bioactive ingredients, especially capsaicin from chilli pepper and green tea, have gained much attention due to their thermogenic properties, which have been shown to increase energy expenditure via stimulation of SNS ^{44,177,191-194,198,199,218}. Furthermore both short-term and long-term studies have indicated that capsaicin affects substrate oxidation in favour of fat oxidation ^{177,193,194,201}. A few studies have revealed that capsaicin suppressed appetite and reduced energy intake due to stimulation of SNS in the short-term whereas no effects were observed on regulation of appetite and energy intake during long-term administration of capsaicin ¹⁹⁸⁻²⁰¹. The energy suppressive effects of capsaicin were most evident at the maximum tolerable dose of chilli pepper and when administered orally when compared to gastrointestinal ingestion suggesting that both sensory stimulation as well as gastrointestinal processing are of importance to the energy regulating effects of capsaicin ^{199,200}. However, long term weight loss strategies using oral administration of capsaicin may be difficult to comply with using maximum tolerable doses of capsaicin due to the strong burning sensations in mouth and stomach ^{200,201}. A short-term study was conducted to reveal if chilli pepper and other hot spices such as horseradish, ginger, mustard and wasabi affect energy intake and appetite when added to well-composed meals in tolerable amounts ²⁷⁸. Chilli, horseradish, mustard and wasabi had no suppressive effect on energy intake and appetite ²⁷⁸. The fact that only minor effects on appetite were found by adding hot spices to the meals suggests that the observed effects most likely could be explained by degree of familiarity with the hot spices as well as their influence on sensory properties of the meals. Based on these results it was

concluded that hot spices used in tolerable doses might have no evident suppressive effects on appetite and energy intake during a single meal occasion. Higher doses or several daily exposures of these hot spices might be needed to obtain beneficial effects on appetite and energy intake. No evidence for thermogenic activity has been found for ginger in humans³⁰¹ and to my knowledge thermogenic properties of horseradish and mustard has not yet been revealed in humans. However, the effects of ginger, black pepper, horseradish and mustard on appetite, energy expenditure and energy intake have recently been investigated³⁰² and the so far unpublished results from this study might reveal if allylisothiocyanates from horseradish, mustard and wasabi, gingerols (6-gingerol, 8-gingerol and zingerone) and shogaols from ginger as well as piperine from black pepper affect energy metabolism, appetite and energy regulation in humans when ingested during a single breakfast meal. Based on these results, hot spices with beneficial effects could be selected for future experiments focusing on the long-term effects of these spices. However, with the current knowledge we decided that the beneficial effects on appetite and energy intake might more likely be found when bioactive ingredients are administered in doses higher than what is normally consumed in a meal. Since current research has focused on effects of green tea on energy expenditure, fat oxidation and weight regulation we found it of interest to estimate whether green tea in addition to the thermogenic effects has energy intake and appetite regulating effects²¹⁸. Different herbal supplements were administered in capsules (capsaicin, CH-19 sweet pepper and placebo), as beverages (green tea) or both (capsaicin + green tea) with three daily meals to study the acute effects of one day exposure to these active compounds on subjective appetite and energy intake²⁷⁹. Subjects were studied under conditions of positive or negative energy balance obtained by manipulating breakfast and lunch sizes whereas a free-access dinner meal was provided to measure energy intake²⁷⁹. The major conclusions were that CH-19 sweet pepper and the combination of green tea and capsaicin induced energy intake suppressive effects, only under conditions of positive energy balance²⁷⁹. Therefore it appears that during positive energy balance energy intake may be easier reduced due to treatments than during negative energy balance, which may explain the failure of possible treatment induced energy intake effects in many weight loss studies. Furthermore these and other findings suggest that CH-19 sweet pepper could be an attractive alternative to capsaicin due to its ability to promote energy expenditure and to suppress energy intake in the short term and enhance fat oxidation in the longer term in humans^{209,211,279}. Interestingly, capsaicin and green tea also affected several of the appetite scores (hunger, fullness and satiety) and

combining capsaicin and green tea caused even greater effects on appetite, yet stronger in negative than in positive energy balance ²⁷⁹. In accordance with these findings capsaicin has previously been found to affect appetite (hunger, satiety) and energy intake in the short term ^{198,200}. The suppressive effects on hunger as well as protein and fat intake were larger with HF meals compared to HC meals ¹⁹⁸. Our findings suggest that capsaicin, green tea and the combination of the two affect appetite in beneficial ways that could lead to negative energy balance when herbal supplementation is maintained for longer periods. Long term administration of green tea was, however, previously found to stimulate EE and to reduce body fat and weight when consumed with the usual diet or with 90 % of the individual energy need ^{212,216}. After weight loss neither green tea nor capsaicin administration improved weight-maintenance and prevented weight gain in the long-term even though fat oxidation and REE were increased with capsaicin and moreover body weight decreased in low caffeine users after long-term green tea administration ^{201,215,218}. Furthermore no anti-obesity effects were observed when green tea was added to a long term LED ²¹³. The present study is one of the few that has shown appetite and energy intake suppressing effects of green tea in humans when administered alone and in combination with other bioactive ingredients ^{213,279}. The fact that combining bioactive ingredients induce greater appetite effects than any of the compounds taken alone is in accordance with several studies reporting promising effects of combinations of bioactive ingredients on energy intake, energy expenditure or fat oxidation in the short term ^{44,279,303-305} whereas long term supplementation moreover improved weight loss ^{177,194,306-309}. Based on recent and previous results we therefore suggest that the appetite suppressive effects of the bioactive ingredients might depend on energy balance and that these ingredients should be used in combination to reveal if they affect appetite, energy intake and body weight in the long term. Thus, future experiments could ideally be conducted by administering herbal supplementation to overweight subjects in the long-term to reveal if capsaicin, green tea, CH-19 sweet pepper or combinations of these food ingredients can contribute significantly to prevention or treatment of overweight and obesity.

Desires and liking of food are important for our food choices and food intake. Therefore we found it of interest to investigate how hot spices affect liking and desire to eat sour, sweet, fat, bitter, salty and hot stimuli during a meal. Interestingly, chilli was found to increase the desire to eat sweet foods whereas mustard decreased the desire for salty stimuli when compared to the non-spicy

fixed meal at a single meal occasion ²⁷⁸. Furthermore, capsaicin, green tea and the combination of the two significantly reduced desires to eat fatty, salty and hot when administered three times daily ²⁷⁹. Additionally, capsaicin reduced desire to eat sour whereas the combination of capsaicin and green tea reduced desire to eat bitter over a day. These findings indicate that bioactive ingredients can change food desires. Capsaicin has previously been found to increase perceived oiliness of the high carbohydrate meals ¹⁷⁷, which could suggest that hot spices affect fat perception and thereby might change desires for fatty stimuli too. The fact that oral burn interacts with gustatory and olfactory stimuli and that these sensory inputs are integrated with hedonic and rewarding responses in the brain, possibly in OFC, could explain why hot spices and green tea might affect sensory specific desires. Liking for well-composed dinner meals was not affected by adding chilli, horseradish, mustard and wasabi to the meals ²⁷⁸. However, mustard increased the liking of the meal without increasing energy intake supporting that the amount of food eaten is relatively consistent regardless of liking ^{79-81,278} or that the expected increase in energy intake due to increased liking could be prevented by energy intake suppressive mechanisms excited by mustard. Furthermore, liking over the meals supplemented with capsaicin and green tea was significantly reduced suggesting that these bioactive components might contribute to stronger SSS, which might lead to a smaller energy intake as well ²⁷⁹. Capsaicin has furthermore been suggested to affect hedonic processing and reward by stimulating release of endorphins, which might result in a kick or rush-like experience ¹¹². We therefore hypothesized that the general mood and well-being might be affected by adding hot spices to the meals. However, horseradish, ginger, mustard and wasabi had only minor effects on general mood. Unfamiliarity with the hot spices and oral burn, especially from ginger and wasabi, as well as changes in liking by adding mustard and finally the fact that hot spices provide a the more intense sensory experience could all have influenced the general mood and well-being ²⁷⁸. We failed to show any effects of chilli on general mood and based on these findings we suggest that hot spices used in tolerable doses have no evident effects on general mood during a single meal occasion ²⁷⁸.

4. Which physiological mechanisms can explain effects of hot spices on energy intake and appetite?

The promising effects of bioactive ingredients such as caffeine, catechins and capsaicin on body weight regulation, satiety, energy-metabolism and body

composition are believed to be caused by their ability to interfere with the activity of the SNS by modulating catecholamine release and activity^{44,46-48,310}. The fact that capsaicin can increase EE by enhancing catecholamine release and that the EE stimulating effect of capsaicin is inhibited by β -blockers suggest that capsaicin increases the activity of SNS through adrenergic stimulation^{177,194}. In green tea, catechins are thought to inhibit catechol O-methyl-transferase (COMT), an enzyme that degrades noradrenalin³¹¹. Furthermore, caffeine in green tea might inhibit the phosphodiesterase-induced degradation of intracellular cyclic AMP (cAMP), a mediator for the action of catecholamine's on thermogenesis and prevent suppression of noradrenalin release by antagonizing adenosine receptors⁴⁴. The effects of catechins and caffeine all together result in increased concentrations of noradrenalin and thus increased stimulation of β -adrenergic receptors leading to increased thermogenesis and fat oxidation^{48,312,313}. CH-19 sweet pepper can also stimulate SNS activity in humans, to a similar extent as chilli peppers²¹⁰. In animals, capsiate administration significantly decreased serum TG and increased concentration of serum FFA and glucose, which further suggests that stimulation of the SNS, mediated via adrenalin release, promotes substrate oxidation by enhancing lipogenesis in the liver and adipocytes as well as the glycogenolysis in the liver²⁰⁶.

Generally it has been found that the activity of the SNS increases during positive energy balance and decreases during negative energy balance, which suggests that SNS has an important role in counteracting positive energy balance to maintain energy balance in humans^{46,314-316}. Based on these proposed mechanisms we hypothesized that the appetite and energy intake regulating effects of hot spices and green tea might depend on the energy balance under which the active compounds are given and we expected effects of these compounds to be greater during positive than negative energy balance. This hypothesis was nevertheless not completely confirmed. Suppressing effects of CH-19 sweet pepper and the combination of capsaicin and green tea were independent of energy balance but were as expected greater during positive energy balance. Appetite regulating effects of capsaicin, green tea and the combination of the two were however dependent of energy balance with larger effects observed during negative energy balance when compared to positive energy balance. These findings suggest that subjects on a LED might benefit from the appetite stimulating effects of bioactive ingredients, however, these effects are not strong enough to limit energy intake further whereas people with tendency towards overeating might benefit from bioactive ingredients

due to their ability to limit energy intake. Therefore, negative and positive energy balances may play a role in how capsaicin, green tea, CH-19 sweet pepper and combination of capsaicin and green tea affect appetite. In the present study we failed to show that capsaicin, green tea, CH-19 sweet pepper and the combination of capsaicin and green tea affect SNS activity using heart rate as a measure, suggesting that catecholamine release rather than heart rate should be preferred as a measure of SNS activity in future studies. However, we suggest that the energy intake suppressive effects of CH-19 sweet pepper, the combination of capsaicin and green tea as well as the appetite regulating effects of capsaicin, green tea and the combination of the two, obtained in our recent study, are mainly caused by stimulation of SNS ²⁷⁹. Furthermore capsaicin but not CH-19 sweet pepper affected appetite ²⁷⁹. Capsaicin and capsiate have previous been shown to promote body temperature, adrenalin and oxygen consumption as well of timing of these responses in animals differently and it was proposed that different absorption processes of the two compounds in the GI tract could be the main cause for these discrepancies ^{206,207}. In humans capsaicin promoted thermogenesis to a greater extent than CH-19 sweet pepper and only capsaicin affected BP and HR ²¹⁰. Therefore, capsaicin might have stronger effects on SNS than CH-19 sweet pepper at the concentrations used in these studies. Alternatively, pungency, different absorption of the two compounds or other unknown mechanisms might affect thermogenesis, BP and HR ²¹⁰. Studying how capsaicin, capsiate and catechins are absorbed, metabolized and excreted might help elucidate the bioactive mechanisms of these ingredients ²¹⁷. NMR-based metabonomics has so far been performed on green and black tea and this approach might be valuable to obtain greater knowledge on how bioactive ingredients affect energy metabolism and biosynthetic pathways ³¹⁷.

In summary, the effects of capsaicin and green tea on appetite and energy intake have only been proven to be short-term effects ^{200,201,279}. We suggest that future studies should investigate how these and other bioactive ingredients individually and in combination affect appetite and energy regulation in the long term. Especially, it would be of great interest to understand to which degree sensory stimulation from hot spices and green tea, gastrointestinal stimulation, as well as energy balance, contribute to the thermogenic properties as well as the appetite and energy regulating effects of these ingredients. The fact that beneficial effects of the bioactive ingredients might diminish in the long term due to habituation might be highly relevant to consider too, since high habitual caffeine intake has led to such

tolerance, making the anticipated effects of caffeine in green tea supplement ineffective^{213,215,218}. The current research has mainly focused on how subjective appetite is influenced by bioactive ingredients such as capsaicin and green tea^{198,200,279}. Subjective appetite is guided by several unconscious and continuous psychological and physiological elements such as desires, hunger, satiety and hedonic sensations and eating behaviour; however, the subjective nature of appetite sensations can make it difficult to determine the accuracy and reproducibility of the method²³⁵. Subjective measures of appetite could therefore beneficially be combined with objective methods measuring energy expenditure, blood parameters and energy intake to fully reveal how appetite regulation is affected by bioactive ingredients. More knowledge might be gained from studying how bioactive ingredients affect the entire satiety cascade. The sensory properties of bioactive ingredients and the active compounds themselves might stimulate a wide array of physiological and metabolic processes, such as excretion of saliva, satiety signals such as CCK, GLP-1, SP and PYY and long term regulators such as leptin that might affect central neural processing in brain areas such as NTS, OFC or hypothalamus, involved in short and long term regulation of appetite and energy intake, taste and flavour perception, as well as eating behaviour. In the present PhD-project it was originally planned to study if capsaicin affects the release of satiety hormones, but lack of time and money prevented these studies from becoming a reality. Furthermore, investigations of thermogenic as well as appetite and energy regulating properties of allylisothiocyanates from horseradish, mustard and wasabi, as well as for gingerols and shogaols are still an unexplored field which might be worthwhile paying more attention to.

Overall, the discussion given above suggest that bioactive ingredients such as capsaicin, green tea and CH-19 sweet pepper possess thermogenic properties as well as appetite and energy intake regulating effects, however, it is questionable whether these effects are large enough to affect body weight in the long term. Healthy eating behaviour as well as an active lifestyle might still be the main success criteria needed to prevent and treat obesity. Hot spices and other thermogenic ingredients might therefore be excellent supplements to common strategies aiming at changing eating behaviour. Furthermore, direct cognitive control of behaviour might be needed to initiate such changes in lifestyle as well as greater awareness of the consequences of eating highly palatable fatty foods. Greater availability of healthier and tastier foods in canteens and restaurants may be one way of helping

consumers to better eating behaviours. However, a deeper insight into how genetics, metabolic (thermogenesis/energy metabolism) and physiological mechanisms (appetite and energy control) interact with environmental factors (social and cultural behaviour, context, cultural defined portion sizes) is needed to understand eating behaviour and genesis of obesity. Genetic variation in taste, such as sensitivity towards PROP, has been proposed to affect dietary intake. Evidence for such connections is still weak and further studies could reveal if genetically determined sensitivity of taste, smell and irritants influence energy intake and food choices^{70,104,120,131-133}. Furthermore, it is still not well understood how the sensory properties of foods, as well as desires, SSS, hedonic sensation and the reward value of pleasurable tastes and flavours affect appetite, energy intake, digestive behaviour and food choices. Further investigation could reveal if flavour, tastes and irritants affect regulation of appetite and energy intake. Greater understanding of how the senses interact and how food components affect appetite and desires would be useful in developing tastier and more satiating foods.

Conclusion

Hot spices are believed to enhance overall flavour of foods; however, research has confirmed that trigeminal stimuli affect taste perception whereas the influence on flavour perception is less explored. We discovered that chilli suppressed meat flavour in chilli spiced pork patties, which suggests that capsaicin suppresses flavour in solid foods. Based on current knowledge we believe that suppression of flavours and tastes are caused by interaction between gustatory, olfactory and trigeminal stimuli however further investigations are needed to reveal if interactions occur via a central neural integration of sensory input and/or via peripheral effects. It has been suggested that oral burn increases linearly with temperature. We found, however, a non-linear temperature dependency of oral burn, since pork patties served at 38 °C were slightly more burning than at 67 °C and least burning at 8 °C. We suggest that the taste and texture complexity of the pork patties have reduced the impact of temperature on oral burn or that binding of capsaicin to its receptor might be temperature dependent with an optimum around 37 °C. Little is known about how bioactive ingredients affect appetite and energy intake. We found that hot spices used in tolerable doses had no evident effects on appetite, general mood and ad libitum energy intake when added to a single well-composed meal. However, three daily exposures of bioactive ingredients revealed that CH-19 sweet pepper and the combination of green tea and capsaicin induced energy intake suppressive effects under conditions of positive energy balance. Interestingly, capsaicin and green tea also affected appetite and combining capsaicin and green tea induced greater satiety and less hunger than when administered individually; yet stronger effects in negative than in positive energy balance were observed. Therefore, we suggest that appetite suppressive effects of bioactive ingredients might depend on energy balance. Bioactive ingredients should be studied further, individually and in combination, to reveal if they affect appetite, energy intake and body weight in the long term and by which mechanism these effects can be explained. Finally, hot spices were found to affect food desires whereas capsaicin and green tea increased SSS. A better understanding of how hot spices affect the overall flavour of foods and how sensory properties of spicy foods affect food desires, appetite, energy intake and digestive behaviour would be useful in developing tastier and healthier foods with greater satiating and satisfying properties.

Future Perspectives

Going through the results obtained during my PhD I feel like I have served the appetizer and that the remaining ideas are ingredients of the main dish that were never served. The following research questions for future studies are extracted from the discussion and are generated from our results as well as those from others.

1. How do hot spices affect overall flavour of foods?

Investigations of how tastes, flavours and irritants interact and how they contribute to overall flavour of food are needed. It should be clarified if possible interactions between senses occur on taste cell level and if chemical interaction between food components affect oral burn and total flavour perception of food. Especially it would be interesting to study the influences of texture, temperature, food matrix, fat and particle size of hot spices on oral burn and overall flavour of foods.

2. How does sensory perception of hot spices affect energy intake and appetite?

More studies should explore how sensory perception of flavour, tastes and irritants affect regulation of appetite and energy intake as well as establish the importance of genetically determined sensitivity towards taste, smell and irritants for sensory perception, energy intake and food choices. It could also be of interest to study if bioactive ingredients can modulate desires and SSS since such effects might influence energy intake and food choices. Especially, capsaicin's ability to reduce desire for fat and increase the perceived fat level should be investigated further to clarify the potential for developing more satisfying low fat meals with hot spices.

3. Do horseradish, ginger, mustard and wasabi in foods affect appetite, energy intake and EE in the short term?

Varying doses and number of exposures of hot spices would be relevant in studies exploring the effects of hot spices on energy intake and appetite regulation as well as energy metabolism in real foods.

4. Do bioactive ingredients in foods have long term effects on appetite, energy intake and body weight?

It would be relevant to study if beneficial effects on appetite and energy intake can be achieved with several exposures of bioactive ingredients when used in tolerable doses and ingested with daily meals. Effects should be studied with bioactive ingredients administered individually and in combination. The influence of energy balance and habituation on energy intake and appetite regulating effects of bioactive ingredients would be relevant to examine, when evaluating the potential of bioactive ingredients for prevention or treatment of overweight and obesity. Greater understanding of how bioactive ingredients affect energy intake, appetite and body weight regulation might be gained from measuring important short term and long term appetite and body weight regulating hormones, as well as neural processing in the brain. The potential of CH-19 sweet pepper as an energy suppressive agent should be exploited further.

5. How do hot spices affect energy metabolism?

Approaches such as NMR-based metabonomics might be useful in revealing how bioactive ingredients are absorbed, metabolized and excreted as well as how they influence energy metabolism. Furthermore, it would be interesting to investigate if lipophilic bioactive ingredients such as capsaicin and catechins affect fat absorption in the gut?

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